

Spatial Transcriptomics Reveals Location-Specific Tumor Cell Subtypes and Signaling within Multifocal Small Intestinal Neuroendocrine Tumors

Akitada Yogo^{1,2}, Naoki Akanuma³, Grace E. Kim^{2,4}, Netta Mäkinen^{5,6}, Chrissie Thirlwell⁷, and Eric K. Nakakura^{1,2}



ABSTRACT

Purpose: Small intestinal neuroendocrine tumors (SI-NET) frequently present as multifocal lesions, but the molecular mechanisms underlying their development and heterogeneity remain unclear. This study aimed to characterize the phenotypes of tumor cell populations across anatomic sites in patients with multifocal SI-NET and identify local microenvironmental factors influencing tumor development.

Experimental Design: Spatial transcriptomics was performed on 72 tissue microarray cores derived from 4 patients with multifocal SI-NETs that included tumoral and nontumoral tissues from various anatomic layers of the small intestine and regional metastatic sites. Unsupervised clustering, overrepresentation analysis, and ligand–receptor (L–R) pair analysis were used to define the tumor cell subtypes and associated signaling networks. External datasets were used for validation. Protein expression of selected genes was evaluated by immunohistochemistry and immunofluorescence.

Results: Unsupervised clustering revealed four major tumor cell subtypes: “mucosal,” “mesenteric,” “lymphatic,” and “deep,” based on their anatomic location and transcriptomic profiles. Each subtype exhibited distinct gene expression patterns and L–R interactions. The “mesenteric” and “lymphatic” subtypes exhibited distinct L–R pairs, such as *NRG1-ERBB3 (HER3)* and *CXCL12-CXCR4*, respectively. *5HT-HTR1D* was found in all subtypes except “mucosal.” Across the four subtypes, *SST-SSTR1/2*, *PTN-NCL*, *MDK-NCL*, and *GJD2-GJD2* were consistently detected, suggesting fundamental roles in SI-NET biology.

Conclusions: Although further validation is needed, our findings indicate that multifocal SI-NETs consist of spatially distinct tumor cell subtypes affected by local cellular interactions, providing insight into SI-NET intratumoral heterogeneity, possible microenvironment-triggered tumorigenesis, and potential subtype-targeted therapeutic strategies.

Introduction

Small intestinal neuroendocrine tumors (SI-NET) are the most prevalent malignancies of the small bowel and are frequently characterized by their multifocal presentation (1, 2). Multifocal SI-NETs have been suggested to develop from clonally independent cells, potentially driven by local tumor-priming factors rather than shared driver mutations (3, 4). However, these location-specific molecular and microenvironmental factors remain systematically unexplored.

Clinically, SI-NETs are notable for their secretion of serotonin and other bioactive compounds, often leading to carcinoid syndrome characterized by flushing, diarrhea, and, in chronic cases, valvular heart disease due to serotonin-induced fibrosis (5, 6). Telotristat ethyl, which inhibits tryptophan hydroxylase, a rate-limiting enzyme in serotonin biosynthesis, is often used in combination with somatostatin analogs (SSA) to manage carcinoid syndrome diarrhea (5, 6). Although 17 genes have been identified to produce 14 serotonin receptor subtypes, the identity of serotonin receptors active in SI-NETs remains unknown (7). Additionally, somatostatin receptors (SSTR), especially SSTR2, are frequently expressed and are widely utilized for both diagnostic imaging and targeted peptide receptor radionuclide therapy (6, 8). Despite these well-known clinical features, their location-specific diversity remains poorly understood.

Primary SI-NET cells are found in various anatomic layers of the small intestine, including the mucosa (m), submucosa (sm), muscularis propria (mp), and subserosa. However, their location-specific characteristics and the extent to which local microenvironmental factors influence their identity have not been systematically investigated. Importantly, previous bulk RNA sequencing (RNA-seq) studies of primary and/or metastatic tumors from patients with SI-NET did not collect tumoral tissue from various anatomic layers within the small intestine, potentially overlooking location-specific expression differences (9–11). We hypothesized that tumor cells possess location-specific phenotypic characteristics influenced by interactions with their spatially distinct microenvironments.

Spatial transcriptomics provides a powerful tool to interrogate gene expression while preserving the spatial context of the tissue architecture. In this study, we employed spatial transcriptomics to

¹Division of Surgical Oncology, Section of Hepatopancreaticobiliary Surgery, Department of Surgery, University of California, San Francisco, San Francisco, California. ²Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California. ³Department of Pathology and Laboratory Medicine, University of California Davis, Sacramento, California. ⁴Department of Pathology, University of California, San Francisco, San Francisco, California. ⁵Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts. ⁶Cancer Program, Broad Institute of Harvard and MIT, Cambridge, Massachusetts. ⁷Bristol Medical School, University of Bristol, Bristol, United Kingdom.

Corresponding Author: Eric K. Nakakura, Department of Surgery, University of California, San Francisco, 550 16th Street, 6th Floor, San Francisco, CA 94158. E-mail: Eric.Nakakura@ucsf.edu

Clin Cancer Res 2026;32:2098–109

doi: 10.1158/1078-0432.CCR-25-2854

This open access article is distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.

©2026 The Authors; Published by the American Association for Cancer Research

Translational Relevance

Small intestinal neuroendocrine tumors (SI-NET) frequently manifest as multifocal lesions, but the underlying mechanisms, particularly the role of the local microenvironment, remain poorly understood. This study on spatial transcriptomics of multifocal SI-NETs provides key insights into intratumoral heterogeneity and the potential influence of the local microenvironment. By characterizing tumor cell subtypes based on anatomic location, such as the mucosal layer, mesentery, and lymph node, the current study reveals spatially distinct gene expression patterns and ligand–receptor (L–R) interactions. Many ligands originate from nontumoral cells, such as somatostatin, semaphorin, and CXCL12, whereas others originate from tumor cells, such as serotonin and Wnt. Strikingly, we identify HTR1D as the serotonin receptor active in enterochromaffin cells and SI-NETs. These findings imply that multifocal SI-NETs within a patient can exhibit location-specific phenotypic diversity influenced by distinct L–R interactions. Such spatially resolved insights may lead to more precise therapeutic strategies and deepen our understanding of disease progression.

investigate potential phenotypic heterogeneity of tumor cells across distinct anatomic sites in patients with multifocal SI-NET. We aimed to determine how tumoral gene expression patterns, including those related to ligand–receptor (L–R) interactions (e.g., serotonin signaling and SSTRs), vary by their anatomic locations within the intestine and to identify local factors that may prime or enhance tumor development.

Materials and Methods

Study design, patients, and Institutional Review Board

Spatial transcriptomics was performed as a retrospective study on 4 patients with multifocal SI-NETs surgically resected at University of California, San Francisco (UCSF) in 2018 and 2022 (Supplementary Table S1). Archived formalin-fixed, paraffin-embedded (FFPE) tissue blocks were selected [9 primary tumors (tp), 4 mesenteric tumors, 2 lymph node metastases, and 6 nontumoral regions]. For additional immunofluorescence (IF) analyses, archived FFPE tissue blocks were selected from 10 patients who underwent surgical resection of primary SI-NETs at UCSF between July 2024 and September 2025. These included 15 tps, 7 mesenteric tumors, 10 lymph node metastases, and 3 liver metastases, comprising 5 patients with multifocal SI-NETs and 5 with unifocal SI-NETs. For the retrospective cohort analysis of serotonin-activating drug use, patients who underwent resection of primary SI-NETs between January 2007 and February 2023 at UCSF were reviewed. The study was performed in accordance with the Declaration of Helsinki and Health Insurance Portability and Accountability Act compliance and was approved by the Institutional Review Board at UCSF. Informed written consent was obtained from each subject. For the retrospective cohort analysis, informed consent was waived because of the retrospective design and the use of anonymous patient data. For external validation, we used the publicly available RNA-seq dataset of human NET, GSE98894, which comprised tumor tissue from 113 NETs from the pancreas, 81 NETs from the small

intestine, and 18 NETs from the rectum (12). RNA-seq datasets of human normal tissues were obtained from the GTEx Portal (version 10 release, RRID:SCR_013042), including small intestine–terminal ileum, colon–sigmoid, pancreas, brain–cortex, and adipose–visceral (omentum) on June 5, 2025 (13). The single-cell RNA-seq dataset of SI-NET (GSM8851402 SiNET1 and GSM8851403 SiNET2 from the series GSE292163) was obtained from the NCBI Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE292163>) on September 10, 2025 (11).

Tissue preparations

To ensure the RNA quality of the FFPE tissue blocks, the percentages of RNA fragments of >200 nucleotides (DV200) were confirmed to be 51% to 75%, meeting the minimum requirement of at least 50%. For each patient, 18 microarray cores (1 mm in diameter) of both nontumoral and tumoral tissues were collected from the blocks (a total of 72 cores, **Fig. 1**; Supplementary Fig. S1). The former included uninvolved background small intestine (m, sm, mp, and ms), and the latter included tumoral tissue from various locations of tps (e.g., m, sm, and mp) or of metastases [e.g., lymph node metastasis and mesenteric metastasis with and without perineural invasion (pni)]. Lymph node metastases were defined as lesions that had an accompanying capsule and lymphoid tissue of a lymph node, whereas mesenteric metastases were tumor deposits lacking residual nodal architecture.

Spatial transcriptomics

The Visium Spatial for FFPE Gene Expression Kit, Human Transcriptome (10x Genomics, PN-1000338, RRID:SCR_023571), was used according to the manufacturer's instructions to generate cDNA with spatial barcode information, followed by sequencing on NovaSeq X (Illumina, RRID:SCR_024569). Read alignment, filtering, barcode counting, and unique molecular identifier (UMI) counting were performed with the Space Ranger (10x Genomics, version 2.1.1, RRID:SCR_025848) count automatic alignment pipeline with the human genome GRCh38 (refdata-gex-GRCh38-2020-A) and Visium Human Transcriptome Probe Set version 1.0 (Visium_Human_Transcriptome_Probe_Set_v1.0_GRCh38-2020-A.csv) to generate feature-spot matrices.

Data processing

Analyses and representations of the data were performed in R (version 4.1.1, Foundation for Statistical Computing, RRID:SCR_001905) using Seurat (version 4.3.0, RRID:SCR_007322) and ggplot2 (version 3.4.2, RRID:SCR_014601) packages. LogNormalize was used to normalize UMI counts. To differentiate the spots into subtypes, unsupervised clustering was performed with the standard Seurat workflow for each patient. The subtypes were annotated based on the corresponding pathologic image (**Fig. 1B**). To minimize the bias caused by cell type mixtures, quality control was performed on the spots. The tumor gene module scores were calculated by AddModuleScore, with tumor module genes as features. Tumor module genes were extracted in each patient as the top 10 most highly expressed genes in tumor-annotated subtypes compared with nontumor-annotated subtypes (top 10 lowest $p_{val,adj}$ with $avg_{log_2FC} > 0.3$, calculated by FindMarkers, Supplementary Table S2). Thus, spots with tumor gene module scores > 2, 2.5, or 3 were selected as tumoral spots that include mainly tumor cells, and spots with tumor gene module scores < 1.5 or 2 were selected as nontumoral spots that include mainly nontumor cells (Supplementary Fig. S2). These tumoral and nontumoral spots with the expression of

representative housekeeping genes (*ACTB*, *B2M*, *UBC*) >0 were used for further analysis (14, 15). In patient 4, one cluster with low nCount was excluded. The qualities of the selected spots were further confirmed by deconvolution analysis using the STdeconvolve (version 1.3.1, RRID:SCR_025977) R package (Supplementary Fig. S3). For dimensional reduction and cluster visualization, the Uniform Manifold Approximation and Projection (UMAP) technique and DimPlot function were used. Datasets from the 4 patients were integrated using the harmony (version 1.2.3) R package. Cell-cycle phase analysis was performed with the CellCycleScoring function in Seurat.

Overrepresentation analysis

To interpret the different expressional characteristics of the subtypes, the ClusterProfiler R package (version 4.2.2, RRID:SCR_016884) was used to perform overrepresentation analysis (ORA) with an adjusted *P* value and *q* value cutoff of 0.1, using the input differentially expressed genes (DEG) extracted with FindMarkers() with test.use = "MAST" (v1.20.0) by $|\text{avg_log2FC}| > 0.3$ and $p_val_adj < 0.01$. For input gene sets, c5.all.v2023.2.Hs.symbols.gmt and c2.cp.v2023.2.Hs.symbols.gmt were downloaded from MSigDB (16–19). Shared gene sets refer to those commonly detected in multiple patients. Cytoscape (version 3.10.2, RRID:SCR_003032), EnrichmentMap (version 3.5.0, RRID:SCR_016052), and AutoAnnotate (version 1.5.1) were used for the visualization and annotation of the detected gene sets (20–23).

L–R pair analysis

To predict cellular signaling communication, CellChat (version 1.6.1, RRID:SCR_021946), NeuronChat (version 1.0.0), and NICHES (version 1.1.0) R packages were used with default parameters. The CellChat netAnalysis_contribution function was used to calculate the contributions of each L–R pair in CellChat datasets, and commonly appearing L–R pairs among all patients were selected. Contributions in NeuronChat datasets were calculated using manual coding as the mean of the probability values of each L–R pair. NICHES was used to visualize L–R pair signal strength on histopathologic images.

External validation using open-source datasets

To validate the expression of discovered L–R genes in tumor cells, GSE98894 was analyzed (12). It was obtained from the GEO database using the GEOquery (version 2.62.2, RRID:SCR_000146) R package. Gene expression normalization was performed using the edgeR (version 3.36.0, RRID:SCR_012802) R package, in which lowly expressed genes were filtered, and normalized counts were transformed into log₂ counts per million. *P* values were calculated using the stats (version 4.1.1, RRID:SCR_025968) R package, kruskal.test function. To validate the expression of discovered ligand genes in nontumor cells, normalized transcript expression data for normal human tissues were obtained from the GTEx Portal (13). To validate the identified transcriptional intratumoral heterogeneity of SI-NET cells, a previously reported single-cell RNA-seq dataset (GSE292163) was analyzed (11). From the SiNET1 and SiNET2 datasets, clusters with high expression of *TPH1* and *CHGA* were extracted as NET cells and reclustered using the FindClusters() function of Seurat. DEGs were extracted with FindMarkers() using test.use = "MAST." Gene set enrichment analysis was performed with the gseGO() function of ClusterProfiler, with genes ranked by log₂ fold change (avg_log2FC). Kyoto Encyclopedia of Genes and Genomes pathway-based

expression visualization was performed with the pathview R package (version 1.34.0, RRID:SCR_002732).

Immunohistochemistry and IF

Tissue blocks were sectioned at 5 μm thickness, and tissue slides were subsequently stained using standard protocols. The primary antibodies and dilution ratios used were 5HT1D (SAB2900609, Sigma-Aldrich, RRID:AB_3696653, 1:100) and Ki-67 (790-4286, Ventana Medical Systems, RRID:AB_2631262, 1:1) for immunohistochemistry (IHC), and serotonin (ab315150, Abcam, RRID:AB_3697404, 1:2,000), 5HT1D (SAB2900609, Sigma-Aldrich, RRID:AB_3696653, 1:100–1:150), and phospho-p44/42 MAPK (Erk1/2; Thr202/Tyr204; 9101S, Cell Signaling Technology, Inc., RRID:AB_331646, 1:400) for IF. Imaging was conducted in a ZEISS Axio Scan.Z1 whole-slide scanner (ZEISS, RRID:SCR_020927). Images were collected with a ZEISS Plan-Apochromat 20x/0.8 NA (Working Distance = 0.55 mm) M27 objective lens in bright-field mode with a Hitachi HV-F202 camera for IHC and an Orca-Flash 4.0 version 2 CMOS camera (Hamamatsu Photonics K.K.) for IF. Positive and negative cells in IHC were quantified using QuPath (version 0.5.0, RRID:SCR_018257), the "Positive Cell Detection" function, in combination with a trained random trees-based object classifier that specifically selected tumor cells (24). Mean cellular fluorescence intensity was quantified using QuPath by defining smooth muscle cells, fibroblast cells, or adipocytes as a negative control in each field of view, and signal intensities were background-corrected by subtracting the mean fluorescence intensity measured in negative control cells.

Retrospective cohort analysis of serotonin-activating drug use

Data were collected from medical records, preoperative and postoperative imaging, operative reports, and pathologic reports. A total of 169 patients who underwent resection of primary SI-NETs between January 2007 and February 2023 at UCSF were included, provided that both medication history and pathologic or radiologic evaluation of mesenteric metastasis were sufficiently documented. Among them, 30 patients had been treated with selective serotonin reuptake inhibitors (SSRI) or serotonin-norepinephrine reuptake inhibitors (SNRI) before referral. Mesenteric metastasis was identified as tumor deposits lacking residual nodal architecture on histopathologic evaluation or, when surgically unresectable, as mesenteric masses identified by radiologic imaging. The Wilcoxon rank-sum and Fisher's exact tests were used to compare distributions across groups.

Results

Unsupervised clustering by expression data exhibited recurrent and location-specific tumor cell subtypes

Spatial transcriptomic analysis was performed on tissue microarray (TMA) cores from 4 patients with multifocal SI-NETs (Fig. 1A). A total of 8,295 spots were analyzed, with a median of 3,102 genes and 5,944 UMIs detected per spot. Across all capture areas, 16,778 unique genes were identified. Unsupervised clustering was performed for each patient, and the resolved nontumoral cell types included superficial, mid-cryptal, and basal aspects of the m, sm, inner and outer layers of mp, myenteric plexus, subserosa, mesenteric nerve, and stroma, as well as distinct tumoral cell subtypes (Fig. 1B). UMAP visualization highlighted subtype-specific differences in tumor cells across anatomic locations (Fig. 2A; Supplementary Tables S3 and S4; Supplementary Fig. S4). In all 4

patients, tumor cells from metastatic sites, such as the tumor deposits in the mesentery and lymph node metastasis, formed distinct clusters compared with tp cells. These included subtypes of “tumor in mesentery (tm),” “tm pni A,” and “tm pni B” in patient 1; “tm #2” and “tm #2 pni” in patient 2; “tl (tumor in lymph nodes)” in patient 3; and “tl” in patient 4. However, some mesenteric metastatic cells clustered together with tp cells (“tm #1 and tp #1 mp” in patient 2, and “tm and tp #2 sm and perithrombus” in patient 3), suggesting the source of metastasis.

Similarly, several tumoral cells sampled from distinct multifocal tps, separated by 2.0 to 27.5 cm (Fig. 1A), clustered together in each patient, such as “tp #1 m and tp #2 m” and “tp #1 mp and tp #2 mp” in patient 1; “tp #1 m and tp #2 m” and “tp #1 sm superficial and tp #2 sm superficial” in patient 2; “tp #1 m and tp #2 m” in patient 3; and “tp #1 m and tp #2 m and tp #3 m” and “tp #1 sm deep and tp #2 subserosa” in patient 4. Notably among them, tumor cells located within the m sampled from geographically separate primary lesions consistently clustered together in each patient (#1 and #2 in patients 1–3 and #1, #2, and #3 in patient 4), which were not observed in tumor cells located within deeper layers (e.g., sm, mp, and/or subserosa). Based on these patterns, tumor cells were categorized into four major subtypes: three distinct subtypes were “mucosal,” “mesenteric,” and “lymphatic,” and a fourth subtype comprised residual cells termed “deep” (including cells located in the sm, mp, and/or subserosa; Fig. 2A). The “mucosal” subtype consistently clustered together even across different patients (Fig. 2B).

Distinct transcriptomic profiles across tumor cell subtypes

To validate the distinct characteristics of the four tumor cell subtypes (mucosal, mesenteric, lymphatic, and deep), ORA was performed. ORA revealed gene sets commonly upregulated or downregulated across patients: “mucosal,” 14 upregulated and 30 downregulated gene sets; “mesenteric,” 6 upregulated and 2 downregulated; “lymphatic,” 0 upregulated and 32 downregulated; and “deep,” 11 upregulated and 6 downregulated (Fig. 2C; Supplementary Table S5).

The “mucosal” subtype showed upregulated gene sets related to actin filament organization and oxidative phosphorylation in aerobic respiration and downregulated gene sets related to capped mRNA splicing, vesicle exocytosis, trans-network budding, and Golgi transport. The “mesenteric” subtype showed upregulated gene sets related to transcription factor activation, as well as neural-related gene sets (e.g., synapse organization and spinal cord injury response) and downregulated extrinsic apoptotic signaling. The downregulated gene sets within the “lymphatic” subtype encompassed steroid/hormone response, cell adhesion, and extracellular matrix (ECM). The “deep” subtype showed upregulated gene sets related to ECM organization, cell substrate junction, and vacuolar lumen and downregulated gene sets related to the immunoglobulin complex.

Cell-cycle phase gene expression analysis suggested that the “mucosal” subtype harbored a relatively higher proportion of proliferative cells (Fig. 3A). This finding was validated by the increased fraction of Ki-67-positive cells in the “mucosal” tumor cells in comparison with other tumor cell subtypes (Fig. 3B).

Distinct and shared L-R pair patterns across tumor cell subtypes

L-R pair analysis revealed recurrent, subtype-specific interactions that were conserved across patients within each tumor cell subtype.

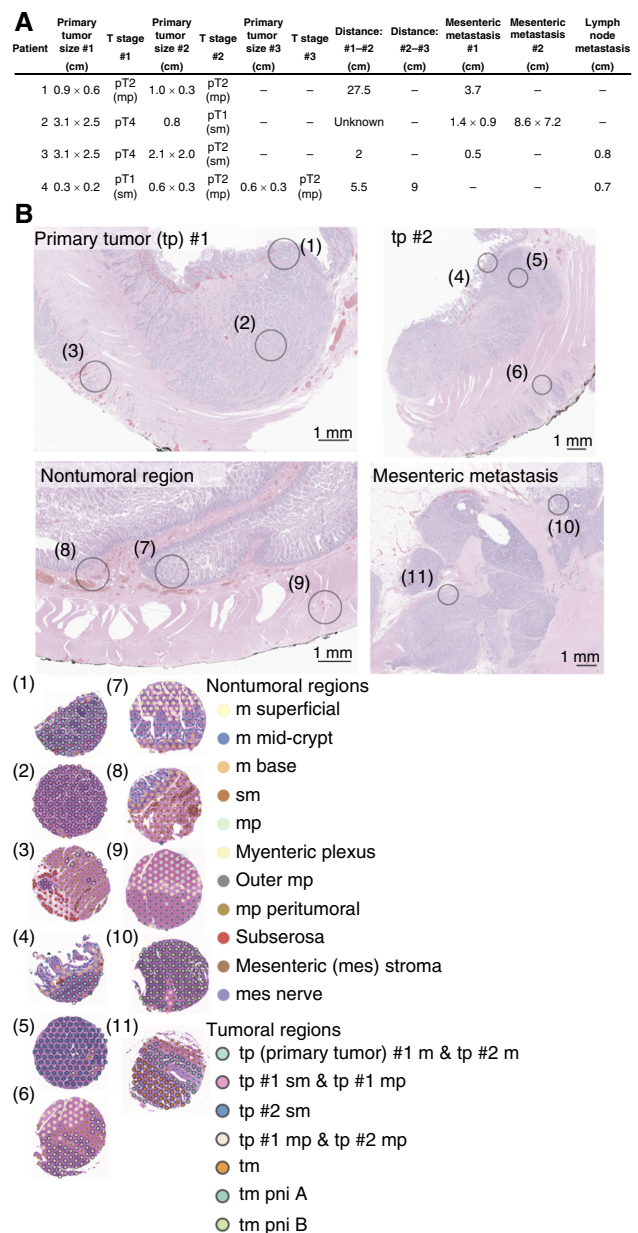
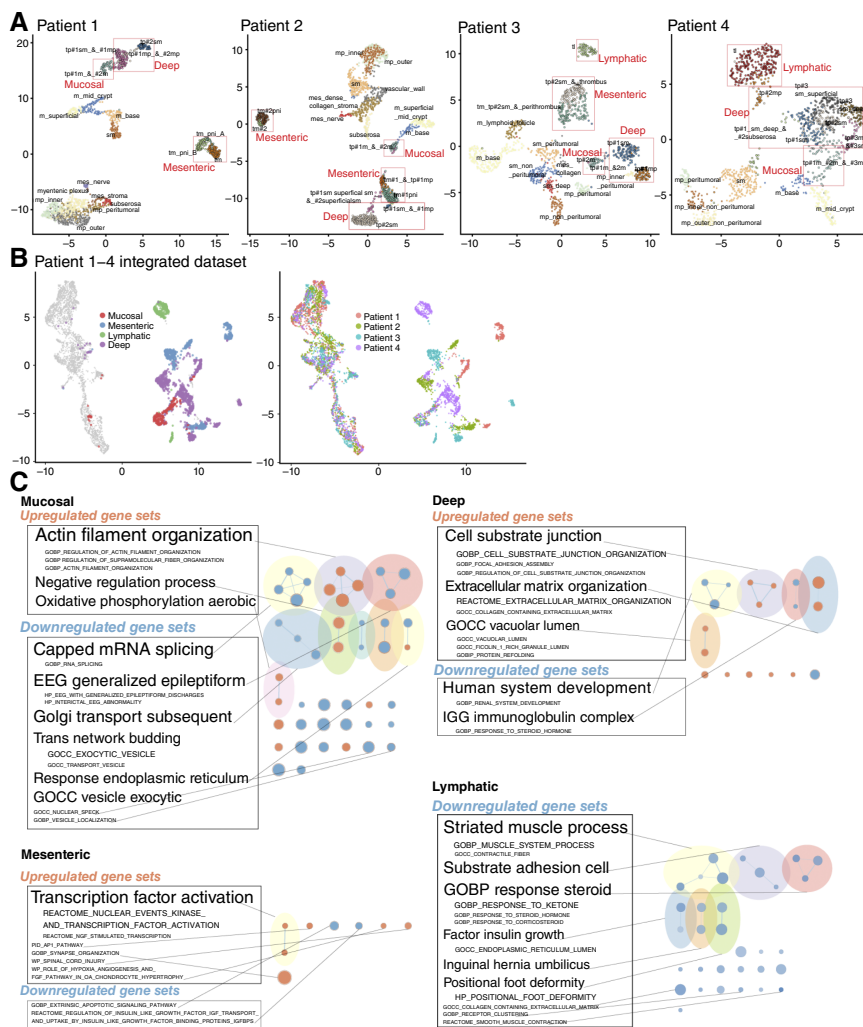


Figure 1.

Overview of samples and spatial transcriptomic annotation. **A**, Table summarizing the clinicopathologic characteristics of the analyzed tumor samples. **B**, Representative TMA cores and spatial spot annotation in patient 1. Circles (1)–(11) indicate the tissue microarrayed cores. A complete set of TMA cores is shown in Supplementary Fig. S1. The suffixes #1 and #2 indicate the different multifocal tumors, whereas A and B indicate distinct cell clusters within the same single tumor.

Among the L-R pairs with ligands originating from nontumoral cells, *PTN-NCL* and *GCG-GIPR* commonly appeared in all the subtypes (Fig. 4A). L-R pairs identified in the “deep” subtype were also present in the “mesenteric” and the “lymphatic” subtypes but were absent in the “mucosal” subtype; these included *PTN-SDC4*, *SEMA3C-PLXND1*, *SEMA3C-(NRP1+NRP2)*, *GUCA2A-GUCY2C*,

**Figure 2.**

Shared and distinct gene signature profiles of tumor cells. **A**, UMAPs showing transcriptionally distinct tumor cell subtypes. **B**, UMAPs based on the integrated dataset from patients 1-4. **C**, Network plots of significantly enriched gene sets shared across patients by subtype. Red bubbles indicate upregulated gene sets; blue bubbles indicate downregulated gene sets. Bubble size corresponds to the number of detected genes per set in patient 1 ("mucosal," "deep," and "mesenteric") and patient 4 ("lymphatic"). The names of the top 10 gene sets with the lowest adjusted *P* value are shown. The full list of the enriched gene sets is provided in Supplementary Table S5.

ADM-CALCR, *TGFB1*-(*ACVR1B*+*TGFB2*), and *TGFB2*-(*ACVR1B*+*TGFB2*).

The "mesenteric" subtype showed distinct L-R pairs such as *NRG1-ERBB3* (*HER3*), *FGF7-FGFR1*, *CSF1-CSF1R*, and *SEMA3B-NRP2+PLXNA2/3*, and the "lymphatic" subtype showed *CXCL12-CXCR4* and *NAMPT-INSR*. Among the L-R pairs with ligands originating from tumoral cells themselves, *MDK-NCL* commonly appeared in all the subtypes (Fig. 4B). Here again, L-R pairs of the "deep" subtype were commonly identified in the "mesenteric" and the "lymphatic" subtypes, but not in the "mucosal" subtype [*NRG1-ERBB3* (*HER3*), *MDK-SDC4*, *MDK-LRP1*, *FGF9-FGFR3*, *FGF9-FGFR1*, and *TGFB1*-(*ACVR1B*+*TGFB2*)]. The "mesenteric" subtype had distinct L-R pairs such as *SEMA3B-NRP1+PLXNA2* and *SEMA3G-NRP2+PLXNA2/3*, and the "lymphatic" subtype had *PDGFA-PDGFRB* and *WNT4/2B-FZD8+LRP5/6*.

When L-R pair analysis specific to neural signaling was performed, the L-R pairs with ligands originating from nontumoral cells, *SST-SSTR1/2*, commonly appeared in all the subtypes (Fig. 4C). *NRXN1/3-NLGN2* was most commonly identified in the "mesenteric," "lymphatic," and "deep" subtypes, but not in the

"mucosal" subtype. Among the L-R pairs with ligands originating from tumoral cells themselves, *GJD2-GJD2* commonly appeared in all the subtypes (Fig. 4D). 5HT (serotonin)-*HTR1D* was most commonly identified in the "mesenteric," "lymphatic," and "deep" subtypes, but not in the "mucosal" subtype (Fig. 4D; Supplementary Table S7).

To validate the expression of potential therapeutic targets, *HTR1D*, *MDK*, *GJD2*, and *NCL* in NETs of other origins and to assess the association of *WNT4* and *CXCR4* expression with metastasis, a publicly available dataset was analyzed (Fig. 4E and F). *HTR1D* expression was distinctively high in SI-NET. *MDK* and *GJD2* showed relatively higher expression in rectal NETs, whereas *NCL* expression was consistent across tumor origins (Fig. 4E). *WNT4* expression was high in metastatic lesions in SI-NET but remained low in liver metastases from pancreatic NETs. *CXCR4* expression was high in lymph node metastases from either SI-NETs or rectal NETs (Fig. 4F).

Expression patterns of ligand genes originating from nontumoral cells, as identified in our dataset, were further examined using external reference datasets (Supplementary Fig. S5). In our data, *PTN*

expression was the highest in the mp, although its median level in the bulk small intestine was approximately 30-fold lower than in the brain cortex, according to external datasets. Similarly, *CXCL12* expression was elevated outside the m in our data, but its median level in the bulk small intestine was about 3.4-fold lower than in the visceral adipose tissue of the omentum.

Serotonin-related heterogeneity and its clinical implications in SI-NETs

To validate the transcriptional heterogeneity of tumoral cell subtypes, especially related to lower serotonin-related expression in the “mucosal” subtype, we analyzed two external single-cell RNA-seq datasets of SI-NET cells. In both datasets, heterogeneity of *TPH1* expression was observed, and the subset with low *TPH1* expression in one dataset exhibited transcriptional features resembling the “mucosal” subtype, characterized by downregulation of signal transportation, along with upregulation of oxidative phosphorylation and cell cycling (Supplementary Fig. S6A and B). Pathway-based expression visualization showed that both the low *TPH1* cells in the external datasets and “mucosal” tumor cells in our dataset exhibited reduced expression of serotonin biosynthetic genes and downregulated Gi/o and MAPK signaling, potential downstream pathways of serotonin receptor HTR1D (Supplementary Fig. S6C).

For orthogonal validation of the heterogeneity of tumoral cells about serotonin-related characteristics at the protein expression level, IHC analysis was performed. First, HTR1D staining on our TMA slides confirmed lower HTR1D protein expression in the “mucosal” subtype compared with submucosal tumor cells (Fig. 5A–D; Supplementary Fig. S7).

Next, we performed serotonin–HTR1D–pMAPK triple IF on our TMA slides. For nontumor tissues, serotonin, HTR1D, and pMAPK fluorescence was detected in cells consistent with serotonin-secreting enterochromaffin cells located in the small intestinal epithelium [Fig. 5E (top)]. Serotonin expression was also detected in the myenteric plexus and mesenteric nerve near tumor cells (Fig. 5F). In contrast, only pMAPK fluorescence was observed in cells consistent with endothelial cells, and no serotonin or HTR1D expression was detected [Fig. 5E (bottom)].

Across the four tumor subtypes, “mucosal” tumor cells exhibited lower mean fluorescence intensities for serotonin, HTR1D, and pMAPK than other tumor subtypes, whereas “mesenteric” tumor cells showed higher serotonin and HTR1D intensities than other tumor subtypes (Fig. 5F and G; Supplementary Table S8). Correlation analysis showed that serotonin and HTR1D fluorescence intensities were positively associated with pMAPK intensity in tumor cells and enterochromaffin cells (tumor serotonin–pMAPK: R^2 0.39, P value < 2.2×10^{-16} ; enterochromaffin serotonin–pMAPK: R^2 0.65, P value = 0.0018; tumor HTR1D–pMAPK: R^2 0.66, P value < 2.2×10^{-16} ; enterochromaffin HTR1D–pMAPK: R^2 0.67, P value = 0.0012), whereas no such correlation was observed in endothelial cells (serotonin–pMAPK: R^2 0.017, P value = 0.23; HTR1D–pMAPK: R^2 0.024, P value = 0.20; Supplementary Fig. S8).

To validate these findings, an additional IF analysis was performed using a separate set of 35 SI-NET specimens, including liver metastases (Fig. 5H; Supplementary Fig. S9; Supplementary Tables S8 and S9). This analysis confirmed lower mean fluorescence intensities for serotonin and HTR1D in “mucosal” tumor cells and higher serotonin and HTR1D intensities in “mesenteric” tumor cells. Liver metastatic tumor cells exhibited lower fluorescence intensities for serotonin, HTR1D, and pMAPK. Notably, heterogeneous serotonin expressions were observed among tumor cells

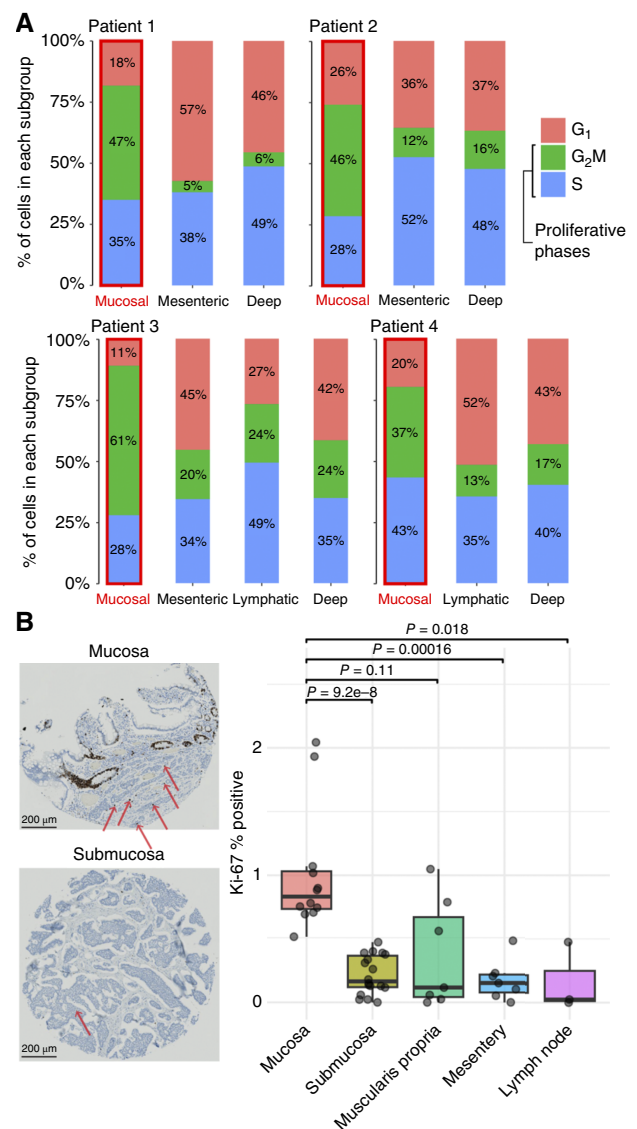


Figure 3.

Elevated proliferative fraction in the “mucosal” tumor cells. **A**, Inferred proliferative states by subtypes based on transcriptional data. **B**, IHC confirms an increased fraction of Ki-67-positive tumor cells in the “mucosal” subtype (top left). The panels to the left show representative images from patient 1 (tumor #1, m, top panel; tumor #2, sm, bottom). Red arrows indicate Ki-67-positive tumor cells. The panel to the right displays a box plot quantifying the percentage of Ki-67-positive tumor cells across anatomic locations in the four patients. Wilcoxon rank-sum test with Bonferroni correction.

within each tumor subtype, with particularly elevated levels in tumor cells located adjacent to mesenteric nerves or at the invasive front (Fig. 5F and I). In addition, mesenteric nerves adjacent to tumor cells, identified in six specimens, exhibited higher serotonin expression than those located distant from tumor cells (Fig. 5I; Supplementary Table S8).

Based on the upregulated serotonin and HTR1D expression in “mesenteric” tumor cells and nerves adjacent to tumor cells, we hypothesized that higher serotonin activity in patients may contribute to

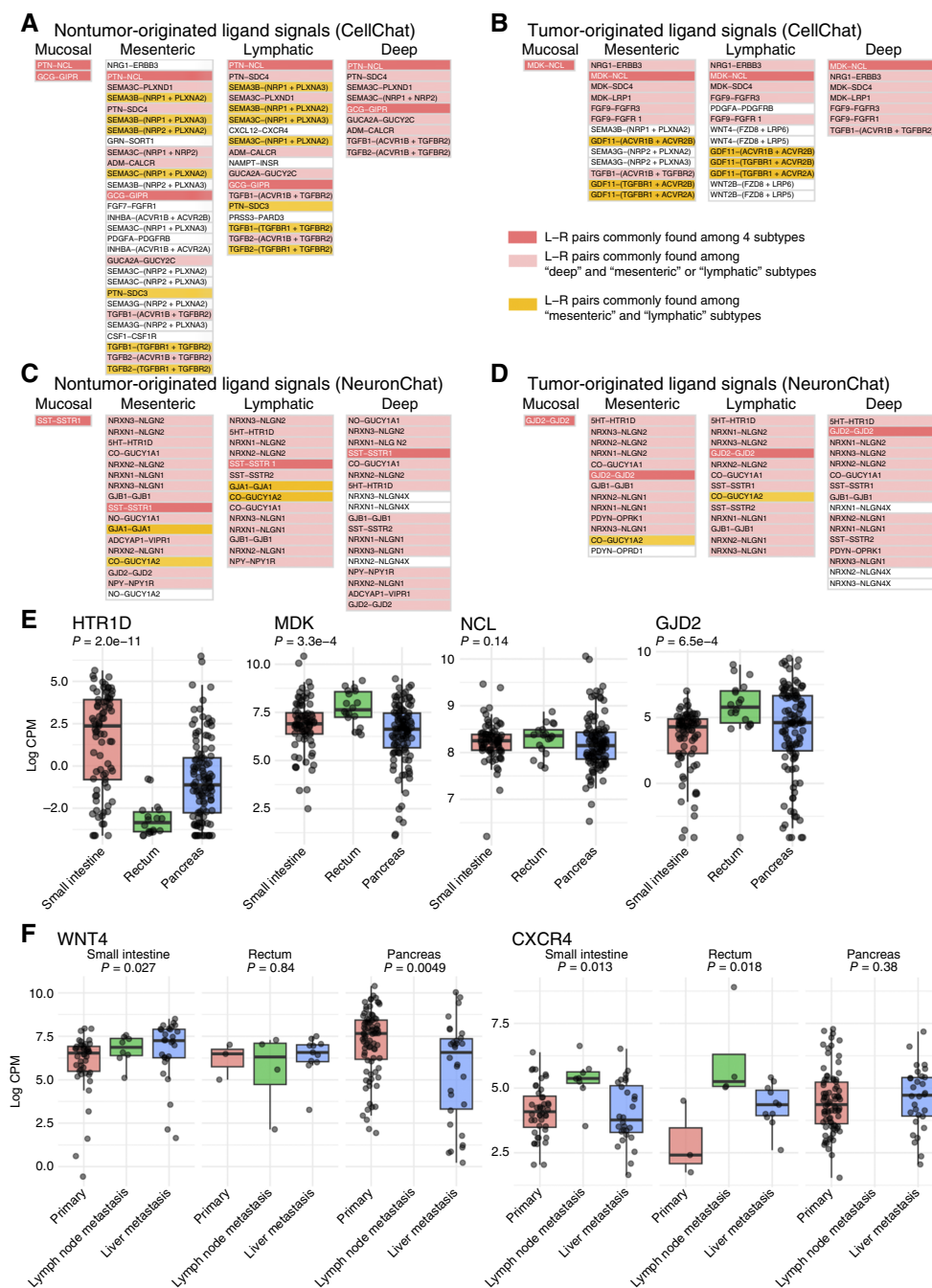


Figure 4. Ranked shared and significant L-R pairs by tumor subtypes across patients. **A-D**, L-R pairs are ranked in descending order based on mean relative contribution ratios within each tumor subtype. The mean relative contribution ratios of the enriched gene sets are provided in Supplementary Table S6. **A** and **B**, Detected using CellChat, showing only those commonly detected in all patients. **C** and **D**, Detected using NeuronChat. Ligands in **A** and **C** originate from nontumoral cells. Ligands in **B** and **D** originate from tumoral cells. **E** and **F**, Box plots of gene expression from GSE98894 with (**E**) expression by tumor origin and (**F**) expression by tumor origin and subtype.

mesenteric metastasis progression. In a retrospective cohort analysis of 169 patients with SI-NET, the use of serotonin-activating drugs, SSRIs or SNRIs, was associated with a higher frequency of mesenteric metastases [97% vs. 75%; OR, 9.7; 95% confidence interval (CI), 1.5–409; $P = 0.0061$], but not with the presence of distant metastasis (50% vs. 58%; OR, 0.72; 95% CI, 0.30–1.7; $P = 0.42$; **Fig. 5J**; Supplementary Table S9).

Discussion

Based on spatial transcriptomics data, this study of multifocal SINETs classified tumor cell subtypes into four categories: “mucosal,”

“mesenteric,” “lymphatic,” and “deep.” Each category exhibited distinct gene expression profiles and a unique hierarchy of L-R pair interactions (**Fig. 6**).

The “mucosal” subtype demonstrated the most consistent gene expression profile both across geographically separate tps within a patient and across different patients. Its enrichment of upregulated gene sets related to actin filament organization and oxidative phosphorylation implies increased proliferation, supported by a higher proportion of cycling cells. In contrast, downregulated pathways involving vesicle transport and Golgi-mediated secretion may reflect its impaired secretory ability. L-R pair analysis revealed

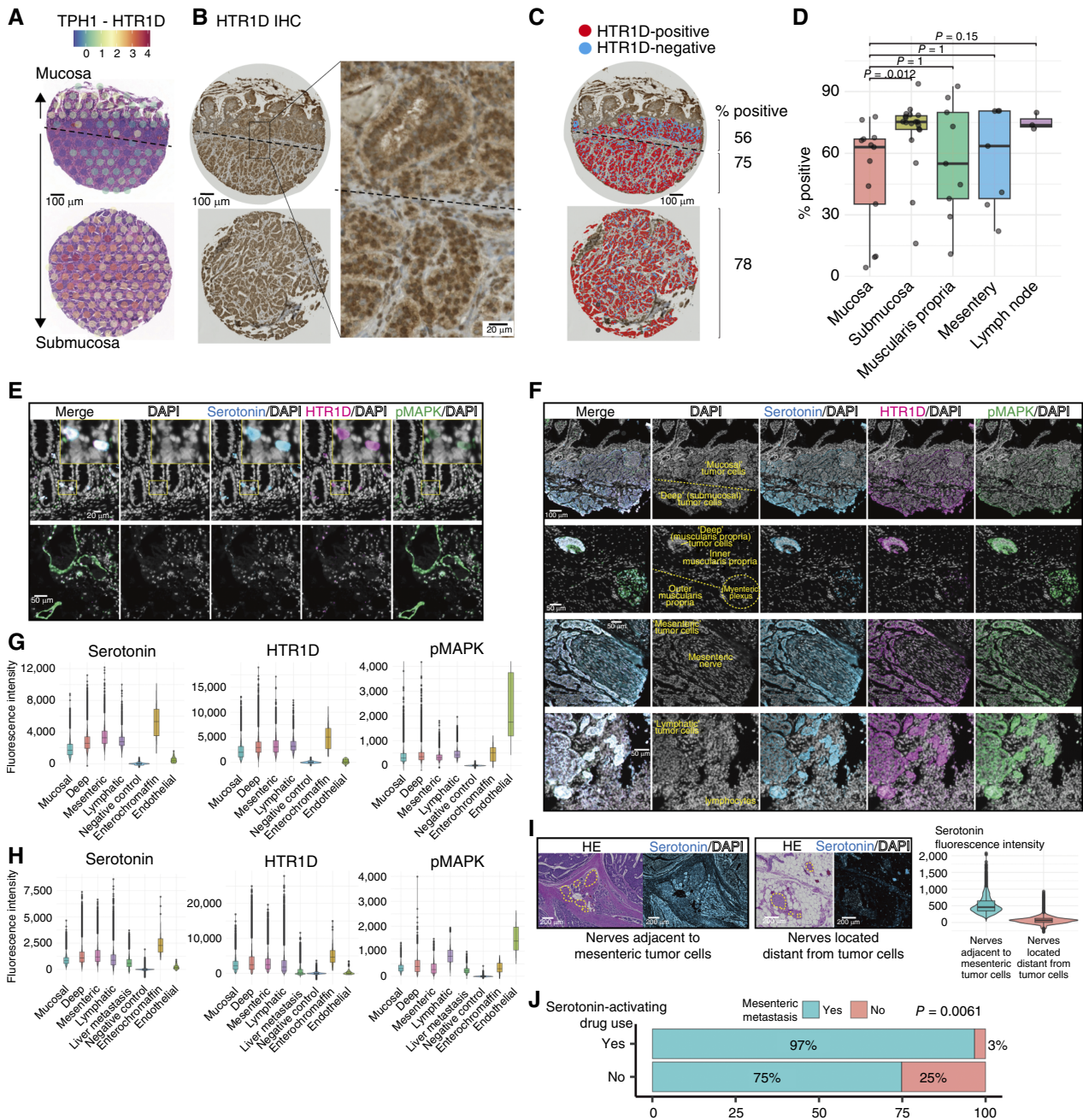
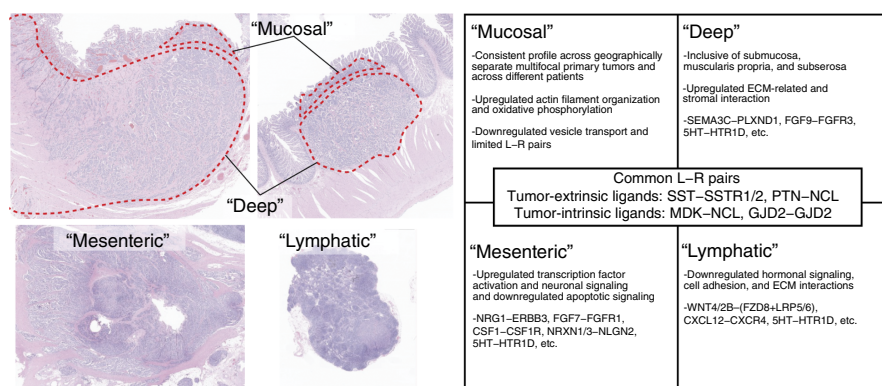


Figure 5.

IHC and IF of serotonin and the serotonin receptor HTR1D. **A–C**, Representative data from patient 4, tps #1 and #3. **A**, Inferred TPH1–HTR1D signaling strength visualized using NICHES overlaid on Visium H&E images. **B**, Corresponding IHC images showing heterogeneous HTR1D protein expression. **C**, Quantification of HTR1D-positive tumor cells, overlaid in each sample (red: positive; blue: negative), with the percentage of positive cells indicated. **D**, Box plots showing the percentage of HTR1D-positive tumor cells across anatomic locations. Wilcoxon rank-sum test with Bonferroni correction. **E** and **F**, Serotonin–HTR1D–pMAPK triple IF images. **E**, Nontumoral regions: crypt level of the m (top) and putative lymphatic ducts in the sm (bottom). **F**, Tumoral regions: mucosal and submucosal tp cells from patient 4 (first row), tp cells invading the mp from patient 2 (second row), mesenteric tumor cells adjacent to nerves from patient 1 (third row), and lymphatic tumor cells from patient 3 (fourth row). **G** and **H**, Box plots and violin plots showing the background-corrected fluorescence intensities of the cells. **G**, Data derived from the four patients included in the spatial transcriptomics cohort. **H**, Data derived from the additional 35 specimens. **I**, Representative H&E images and IF images of nerves adjacent to or distant from tumor cells (left). Nerves are outlined with yellow dashed lines. Box plots and violin plots showing the background-corrected fluorescent intensities of nerve cells identified in six specimens (right). **J**, Bar plots showing the higher frequency of mesenteric metastases progression in patients who had used SSRIs or SNRIs. Fisher’s exact test.

**Figure 6.**

Spatial cell subtype diversity and signaling networks in multifocal SI-NET. Summary of key findings in the current study.

that the "mucosal" subtype shared few L-R pairs with the other subtypes and lacked subtype-specific L-R interactions. Well-established microenvironment-to-tumor L-R pairs such as *PTN-SDC4*, *SEMA3C-(NRP1+NRP2)*, and *GUCA2A-GUCY2C* were not enriched in the "mucosal" group, potentially indicating a reduced reliance on microenvironmental feedback (25–27). Additionally, "mucosal" tumors exhibited lower 5HT-HTR1D interactions, validated by heterogeneous HTR1D protein expression, despite SI-NET's characteristic serotonin production (5). These findings suggest that the "mucosal" subtype may represent a cell-autonomous, metabolically active phenotype with limited microenvironmental dependency.

The "mesenteric" subtype exhibited upregulated gene sets associated with transcription factor activation and neuronal signaling and downregulation of apoptotic signaling. Correspondingly, it exhibited L-R interactions such as *NRG1-ERBB3* (*HER3*), *FGF7-FGFR1*, and *CSF1-CSF1R*, which are involved in growth factor-mediated survival, migration, and neuronal signaling (28–31). Moreover, neuronally relevant L-R pairs, such as *NRXN1/3-NLGN2* and 5HT-HTR1D, were enriched, consistent with our sampling of perineural invasive sites, which likely contributed to the observed L-R pairs. These results suggest a signaling-responsive and apoptosis-resistant tumor phenotype for the "mesenteric" subtype.

The "lymphatic" subtype showed consistent downregulation in pathways linked to hormonal signaling, cell adhesion, and ECM interactions, suggesting a low-adhesion, migratory phenotype. The presence of L-R pairs such as *CXCL12-CXCR4* supports a chemotactic migratory program, frequently implicated in lymphatic spread and distant metastasis in NET and other cancers, and CXCR4 has already been tested in a clinical trial for pulmonary neuroendocrine cancer (32–37). Additionally, the elevated expression of *CXCL12* in the sm and mp in our datasets and notably in visceral adipose tissue of the omentum in external datasets may indicate a directed migration of tumor cells toward these regions. The presence of *WNT4/2B-(FZD8+LRP5/6)* may indicate an autocrine signaling mechanism of tumor microenvironmental factors that contribute to epithelial-mesenchymal transition (EMT), loosening cell adhesion (38).

The "deep" subtype, with tumor cells located within the sm, mp, and subserosa, was enriched for ECM-related and stromal interaction gene sets, consistent with a fibroblast-enriched microenvironment in these regions. Previous studies show cross-talk between tumoral cells and fibroblasts (39). It shared multiple L-R pathways with the "mesenteric" and "lymphatic" subtypes, including *SEMA3C-PLXND1* and *FGF9-FGFR3*, all of which have

established roles in tumor invasion, angiogenesis, and EMT-like behavior (40, 41).

Across the four subtypes, common tumor-extrinsic L-R pairs like *SST-SSTR1/2* and *PTN-NCL* or tumor-intrinsic L-R pairs like *MDK-NCL* and *GJD2-GJD2* may play a fundamental role in their baseline homeostasis or tumoral initiation. The therapeutic effect of inhibiting SST in SI-NET is already validated by clinical SSA use, resulting in hormonal control, antiproliferative effect, and prolonged prognosis (6, 8). In our L-R pair analysis, although *SST-SSTR1* appeared commonly across all the subtypes, *SST-SSTR2* was restricted to the "deep" and "lymphatic" subtypes. This differential L-R pair profile may support the clinical rationale for SSA selection: in SI-NETs that are resistant to standard SSAs such as octreotide and lanreotide, which primarily target SSTR2 and SSTR5, newer multitargeted SSAs like pasireotide, with high affinity for SSTR1, may be an alternative therapeutic strategy (42).

PTN-NCL signaling represents a significant microenvironment-tumor communication pathway that affects cancer progression across multiple tumor types (26, 43, 44). It plays a role in the development and progression of prostate NET and the metastatic potential of pancreatic NET (45, 46). A recent single-cell study of the human nontumoral small intestine highlights *PTN-NCL* involvement with T-cell signaling (47). MDK is a heparin-binding growth factor that has emerged as a key player in tumor cell communication. *MDK-NCL* has been identified as highly enriched in tumor-tumor cell communication in several cancers, functioning via an autocrine signaling mechanism (48, 49). GJD2 encodes connexin-36, a gap junction protein that forms channels between adjacent cells, and is associated with chemotherapy resistance and regulation of apoptosis (50). Moreover, *NCL*, *MDK*, and *GJD2* were consistently expressed across other gastrointestinal/pancreatic NETs, suggesting potential shared functions and therapeutic targets.

In contrast, *HTR1D* expression was distinctively high in SI-NETs, aligning with their autonomous secretion of serotonin, a neurotransmitter and hormone for which *HTR1D* encodes a receptor (5). *HTR1D* is also upregulated in hepatocellular, gastric, and colorectal cancers, in which it promotes tumor growth, migration, and treatment resistance through pathways, such as PI3K/Akt and Wnt/ β -catenin (51–53). Notably, "mucosal" tumor cells exhibited a lower fraction of HTR1D expression, potentially shaping their biological characteristics. To our knowledge, this is the first study to comprehensively assess the possible contribution of each serotonin receptor subtype in SI-NETs using a database of 17 genes producing 14 serotonin receptor subtypes (HTR1A, HTR1B, HTR1D, HTR1E,

HTR1F, HTR2A, HTR2B, HTR2C, HTR3A, HTR3B, HTR3C, HTR3D, HTR3E, HTR4, HTR5A, HTR6, and HTR7).

The “mucosal” subtype is characterized by lower mean *TPH1* and serotonin expression, as supported by IF analysis and partially by external single-cell RNA-seq datasets. However, IF analysis revealed intrasubtype heterogeneity in serotonin expression, and low serotonin expression was also detected in subsets of tumor cells from other subtypes. Therefore, low *TPH1* or serotonin expression alone is insufficient as a definitive marker for the “mucosal-like” subtype.

In contrast, enhanced serotonin activation may support tumor formation in mesenteric metastatic microenvironments, as suggested by our clinical cohort analysis. Our spatial transcriptomics and IF analysis indicated that “mesenteric” tumor cells were the most responsive to neuronal signaling, characterized by upregulated gene sets related to synapse organization, as well as the highest levels of serotonin production and HTR1D expression among the tumor subtypes. Moreover, IF analysis revealed that mesenteric nerves surrounded by tumor cells exhibited higher serotonin expression than those located distant from tumor cells. Similarly, in pancreatic cancer, nerves invaded by cancer cells are associated with a difference in the production of neurotransmitters, suggesting neural remodeling by cancer cells (54). Given that the enteric nervous system is highly responsive to serotonin signaling (55), tumor cells may engage in bidirectional cross-talk with adjacent mesenteric neural cells, leading to neural remodeling. Alternatively, tumor cells may preferentially migrate along serotonin-active nerves, reflecting selective tropism via the identified L–R pair signaling pathways.

HTR1B/D signaling has been reported to promote proliferation in vascular smooth muscle cells via MAPK/ERK signaling (56). Consistent with this, our pathway analysis and IF correlation analysis suggested an association between serotonin or HTR1D expression and MAPK/ERK activation in tumor cells. However, this correlation was modest in “mucosal” and “deep” tumor cells and weak in liver metastatic cells, indicating that other pathways likely contribute to the regulation of MAPK/ERK signaling in these cells. Moreover, other downstream pathways of HTR1D signaling, such as canonical Gi/o signaling and PI3K/Akt or Wnt/ β -catenin pathways, may also contribute to subtype-specific phenotypic properties. Nevertheless, increased serotonin-related activation via HTR1D may serve as a potential biomarker of enhanced regional invasion or mesenteric metastatic potential and as a therapeutic target in SI-NETs.

Previous studies mainly focused on intertumoral heterogeneity; however, the present study highlights intratumoral heterogeneity in SI-NETs. As enterochromaffin cells in the m are considered potential cells of origin for NETs, the distinct and shared characteristics of “mucosal” tumor cells across geographically separate tumors, such as enhanced metabolic and proliferative activity and reduced microenvironmental dependency, may provide key insights into their tumor development (57).

This study has several limitations. First, the sample size of the spatial transcriptomics analysis was relatively small, and all included tumors had low Ki-67 indices (<2%, 0.5%, 0.9%, and 3.3%). Although this allowed for a deep investigation of these well-differentiated SI-NETs, the limited cohort may restrict the generalizability of the findings. To mitigate this, we validated key results using external datasets and additional patient cohorts, which provided support for the robustness and broader relevance of our observations.

Second, the use of FFPE tissue may present challenges due to RNA degradation. Although DV200 values were assessed to ensure

RNA quality, transcriptomic coverage may be biased toward more stable transcripts, potentially affecting the sensitivity of differential expression and downstream analyses. Third, the resolution of the Visium platform was at the level of 55 μ m, and SI-NET tumor cells generally measure about 10 to 20 μ m in diameter and, therefore, are not at the precision of single cells. Although quality control and deconvolution methods were applied to select representative tumoral and nontumoral spots, cellular mixtures within each spot could still confound cell subtype classification and L–R inference.

Fourth, although L–R analyses and ORA provide valuable insights into potential signaling interactions and enriched pathways, these inferences remain exploratory and require further functional validation. Importantly, the L–R analysis did not account for potential somatic mutations or posttranscriptional modifications in receptor genes that may alter receptor functionality. As such, the actual biological activity of predicted L–R interactions may differ from transcriptomic predictions. However, several well-known L–R pairs in SI-NETs, such as CXCL12–CXCR4 and SST–SSTR1/2, were confirmed by our analysis. Moreover, our comprehensive analysis identified HTR1D as the predominant receptor for serotonin in SI-NETs, providing new insights into this important auto/paracrine signaling pathway.

Fifth, the spatial transcriptomic data were not integrated with other omics data like genomics, proteomics, metabolomics, or single-cell RNA-seq. However, we partially mitigated this limitation by validating protein expression of key genes using IHC, providing additional support at the protein level. Finally, although we identified subtype-specific patterns associated with tumor cell location and proposed potential tumor-priming or enhancing factors, the cross-sectional nature of this study limits the ability to infer causality in tumor development. Future longitudinal or functional studies will be necessary to validate our results.

Conclusion

Through the integration of transcriptional profiling and signaling network analysis, this study implies distinct biological programs associated with location-specific cell subtypes in multifocal SI-NETs. Although further validation is needed, these findings offer valuable insights into the mechanisms behind the spatial dynamics of the tumor, potentially guiding subtype-specific diagnostic and therapeutic strategies.

Data Availability

The 10x Visium spatial transcriptomics data generated in this study are available from the NCBI GEO database under accession number GSE301265 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE301265>). The code used for data analysis is available on GitHub: https://github.com/yogo-akitada/SINET_spatial.

Authors' Disclosures

N. Mäkinen reports grants from the Neuroendocrine Tumor Research Foundation during the conduct of the study. C. Thirlwell reports grants from the Neuroendocrine Tumor Research Foundation during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

A. Yogo: Conceptualization, data curation, software, formal analysis, investigation, visualization, methodology, writing—original draft, project administration. **N. Akanuma:** Conceptualization, resources, investigation, methodology, writing—review and editing. **G.E. Kim:** Conceptualization, resources, funding acquisition, investigation, methodology, writing—review and editing. **N. Mäkinen:** Conceptualization,

resources, data curation, funding acquisition, investigation, methodology, project administration, writing—review and editing. **C. Thirlwell:** Conceptualization, resources, data curation, funding acquisition, investigation, methodology, project administration, writing—review and editing. **E.K. Nakakura:** Conceptualization, resources, data curation, funding acquisition, investigation, methodology, project administration, writing—review and editing.

Acknowledgments

This work was supported by the Neuroendocrine Tumor Research Foundation, the Placzek Family Foundation, and Larry and Margaret Hauben. We would like to thank Matthew Meyerson, MD, PhD, Department of Medical Oncology, Dana-Farber Cancer Institute, Cancer Program, Broad Institute of Harvard and MIT, Cambridge, Departments of Genetics and Medicine, Harvard Medical School, for funding acquisition and valuable feedback; Jolene Guerin and Yunn-Yi Chen, MD, PhD, Department of Pathology, University of California, San Francisco, for preparing the TMA block; Yunita Lim and Anny Shai, PhD, UCSF Brain Tumor Center, University of California, San Francisco, for sectioning the blocks; Felicia

Miller, Mylinh Bernardi, and Horng-Ru Lin, PhD, Gladstone Genomics Core, for running the Visium protocol; Ayushi Agrawal, Gladstone Genomics Core, for generating the gene barcode expression matrix and submitting the data to the GEO database; and Kristine Wong, Emanuela Zacco, PhD, and Scott C. Kogan, MD, Department of Laboratory Medicine, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, for immunohistochemistry and immunofluorescence. Generative AI was used to improve the language and readability of the manuscript originally written by the authors.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Received August 11, 2025; revised January 10, 2026; accepted March 2, 2026; posted first March 4, 2026.

References

- Howe JR, Karnell LH, Menck HR, Scott-Conner C. The American College of Surgeons Commission on Cancer and the American Cancer Society. Adenocarcinoma of the small bowel: review of the national cancer data base, 1985-1995. *Cancer* 1999;86:2693-706.
- Bilimoria KY, Bentrem DJ, Wayne JD, Ko CY, Bennett CL, Talamonti MS. Small bowel cancer in the United States: changes in epidemiology, treatment, and survival over the last 20 years. *Ann Surg* 2009;249:63-71.
- Mäkinen N, Zhou M, Zhang Z, Kasai Y, Perez E, Kim GE, et al. Whole genome sequencing reveals the independent clonal origin of multifocal ileal neuroendocrine tumors. *Genome Med* 2022;14:82.
- Elias E, Ardalan A, Lindberg M, Reinsbach SE, Muth A, Nilsson O, et al. Independent somatic evolution underlies clustered neuroendocrine tumors in the human small intestine. *Nat Commun* 2021;12:6367.
- Perez K, Del Rivero J, Kennedy EB, Basu S, Chauhan A, Connolly HM, et al. Symptom management for well-differentiated gastroenteropancreatic neuroendocrine tumors: ASCO guideline. *JCO Oncol Pract* 2025;22:19-35.
- Lamarca A, Bartsch DK, Caplin M, Kos-Kudla B, Kjaer A, Partelli S, et al. European Neuroendocrine Tumor Society (ENETS) 2024 guidance paper for the management of well-differentiated small intestine neuroendocrine tumours. *J Neuroendocrinol* 2024;36:e13423.
- Svejda B, Kidd M, Timberlake A, Harry K, Kazberouk A, Schimmack S, et al. Serotonin and the 5-HT7 receptor: the link between hepatocytes, IGF-1 and small intestinal neuroendocrine tumors. *Cancer Sci* 2013;104:844-55.
- Rogoza O, Megnis K, Kudrjavceva M, Gerina-Berzina A, Rovite V. Role of somatostatin signalling in neuroendocrine tumours. *Int J Mol Sci* 2022;23:1447.
- Andersson E, Arvidsson Y, Swärd C, Hofving T, Wängberg B, Kristiansson E, et al. Expression profiling of small intestinal neuroendocrine tumors identifies subgroups with clinical relevance, prognostic markers and therapeutic targets. *Mod Pathol* 2016;29:616-29.
- Patte C, Pommier RM, Ferrari A, Fei-Lei Chung F, Ouzounova M, Moullé P, et al. Comprehensive molecular portrait reveals genetic diversity and distinct molecular subtypes of small intestinal neuroendocrine tumors. *Nat Commun* 2025;16:2197.
- Somech E, Halder D, Spitzer A, Barbolin C, Tyler M, Halperin R, et al. Subtypes and proliferation patterns of small intestine neuroendocrine tumors revealed by single-cell RNA sequencing. *Elife* 2025;13:RP101153.
- Alvarez MJ, Subramaniam PS, Tang LH, Grunn A, Aburi M, Rieckhof G, et al. A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors. *Nat Genet* 2018;50:979-89.
- GTEX Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* 2020;369:1318-30.
- Gorji-Bahri G, Moradtabrizi N, Vakhshiteh F, Hashemi A. Validation of common reference genes stability in exosomal mRNA-isolated from liver and breast cancer cell lines. *Cell Biol Int* 2021;45:1098-110.
- Wen X, Yang G, Dong Y, Luo L, Cao B, Mengesha BA, et al. Selection and validation of reference genes for pan-cancer in platelets based on RNA-sequence data. *Front Genet* 2022;13:913886.
- Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012;16:284-7.
- Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The molecular signatures database hallmark gene set collection. *Cell Syst* 2015;1:417-25.
- Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545-50.
- Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics* 2011;27:1739-40.
- Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;13:2498-504.
- Merico D, Isserlin R, Stueker O, Emili A, Bader GD. Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. *PLoS One* 2010;5:e13984.
- Kucera M, Isserlin R, Arkhangorodsky A, Bader GD. AutoAnnotate: a Cytoscape app for summarizing networks with semantic annotations. *F1000Res* 2016;5:1717.
- Ortega-Legarreta A, Maillo A, Mouzo D, López-Pérez AR, Kular L, Pahlevan Kakhki M, et al. GeneSetCluster 2.0: a comprehensive toolset for summarizing and integrating gene-sets analysis. *BMC Bioinformatics* 2025;26:219.
- Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: open source software for digital pathology image analysis. *Sci Rep* 2017;7:16878.
- Peng H, Yang M, Feng K, Lv Q, Zhang Y. Semaphorin 3C (Sema3C) reshapes stromal microenvironment to promote hepatocellular carcinoma progression. *Signal Transduct Target Ther* 2024;9:169.
- Sorrelle N, Dominguez ATA, Brekken RA. From top to bottom: midkine and pleiotrophin as emerging players in immune regulation. *J Leukoc Biol* 2017;102:277-86.
- Pattison AM, Merlino DJ, Blomain ES, Waldman SA. Guanylyl cyclase C signaling axis and colon cancer prevention. *World J Gastroenterol* 2016;22:8070-7.
- Wang Q, Wang J, Xu K, Luo Z. Targeting the CSF1/CSF1R signaling pathway: an innovative strategy for ultrasound combined with macrophage exhaustion in pancreatic cancer therapy. *Front Immunol* 2024;15:1481247.
- Vitale G, Cozzolino A, Malandrino P, Minotta R, Puliani G, Saronni D, et al. Role of FGF system in neuroendocrine neoplasms: potential therapeutic applications. *Front Endocrinol (Lausanne)* 2021;12:665631.
- Laskin J, Liu SV, Tolba K, Heining C, Schlenk RF, Cheema P, et al. NRG1 fusion-driven tumors: biology, detection, and the therapeutic role of afatinib and other ErbB-targeting agents. *Ann Oncol* 2020;31:1693-703.
- Chitu V, Gokhan S, Nandi S, Mehler MF, Stanley ER. Emerging roles for CSF-1 receptor and its ligands in the nervous system. *Trends Neurosci* 2016;39:378-93.
- Zhong W, Chen W, Zhang D, Sun J, Li Y, Zhang J, et al. CXCL12/CXCR4 axis plays pivotal roles in the organ-specific metastasis of pancreatic adenocarcinoma: a clinical study. *Exp Ther Med* 2012;4:363-9.

33. Shi Y, Riese DJ, Shen J. The role of the CXCL12/CXCR4/CXCR7 chemokine axis in cancer. *Front Pharmacol* 2020;11:574667.
34. Cives M, Quaresmini D, Rizzo FM, Felici C, D'oronzio S, Simone V, et al. Osteotropism of neuroendocrine tumors: role of the CXCL12/CXCR4 pathway in promoting EMT in vitro. *Oncotarget* 2017;8:22534–49.
35. Sanchis-Pascual D, Del Olmo-García MI, Prado-Wohlwend S, Zac-Romero C, Segura Huerta Á, Hernández-Gil J, et al. CXCR4: from signaling to clinical applications in neuroendocrine neoplasms. *Cancers (Basel)* 2024;16:1799.
36. Ruan C, Zhang J, Liao Y, Hang Y, Dong Y, Shi H, et al. CXCR4 expression in immunohistochemistry of gastrointestinal neuroendocrine neoplasms: a meta-analysis. *J Immunoassay Immunochem* 2025;46:209–17.
37. Fath MA, Liu D, Ewald JT, Robles-Planells C, Tomanek-Chalkley AM, Graves SA, et al. Chemokine receptor CXCR4 radioligand targeted therapy using ¹⁷⁷Lutetium-pentixather for pulmonary neuroendocrine cancers. *Radiat Res* 2024;201:35–47.
38. Yang D, Li Q, Shang R, Yao L, Wu L, Zhang M, et al. WNT4 secreted by tumor tissues promotes tumor progression in colorectal cancer by activation of the Wnt/ β -catenin signalling pathway. *J Exp Clin Cancer Res* 2020;39:251.
39. Cives M, Pelle E, Quaresmini D, Rizzo FM, Tucci M, Silvestris F. The tumor microenvironment in neuroendocrine tumors: biology and therapeutic implications. *Neuroendocrinology* 2019;109:83–99.
40. Miyato H, Tsuno NH, Kitayama J. Semaphorin 3C is involved in the progression of gastric cancer. *Cancer Sci* 2012;103:1961–6.
41. Bhattacharya R, Ray Chaudhuri S, Roy SS. FGF9-induced ovarian cancer cell invasion involves VEGF-A/VEGFR2 augmentation by virtue of ETS1 upregulation and metabolic reprogramming. *J Cell Biochem* 2018;119:8174–89.
42. Melhorn P, Mazal P, Wolff L, Kretschmer-Chott E, Raderer M, Kieseewetter B. From biology to clinical practice: antiproliferative effects of somatostatin analogs in neuroendocrine neoplasms. *Ther Adv Med Oncol* 2024;16:17588359241240316.
43. Lin C, Chen Y, Zhang F, Zhu P, Yu L, Chen W. Single-cell RNA sequencing reveals the mediatory role of cancer-associated fibroblast PTN in hepatitis B virus cirrhosis-HCC progression. *Gut Pathog* 2023;15:26.
44. Koutsoumpa M, Polyarchou C, Courty J, Zhang Y, Kieffer N, Mikelis C, et al. Interplay between $\alpha\beta 3$ integrin and nucleolin regulates human endothelial and glioma cell migration. *J Biol Chem* 2013;288:343–54.
45. Wang J, Zhao F, Zhang Q, Sun Z, Xiahou Z, Wang C, et al. Unveiling the NEFH+ malignant cell subtype: insights from single-cell RNA sequencing in prostate cancer progression and tumor microenvironment interactions. *Front Immunol* 2024;15:1517679.
46. He L, Boulant S, Stanifer M, Guo C, Nießen A, Chen M, et al. The link between menin and pleiotrophin in the tumor biology of pancreatic neuroendocrine neoplasms. *Cancer Sci* 2022;113:1575–86.
47. Gu W, Eke C, Gonzalez Santiago E, Olaloye O, Konnikova L. Single-cell atlas of the small intestine throughout the human lifespan demonstrates unique features of fetal immune cells. *Mucosal Immunol* 2024;17:599–617.
48. Yu X, Xie L, Ge J, Li H, Zhong S, Liu X. Integrating single-cell RNA-seq and spatial transcriptomics reveals MDK-NCL dependent immunosuppressive environment in endometrial carcinoma. *Front Immunol* 2023;14:1145300.
49. Li S, Wang Z, Da HH. Deciphering ovarian cancer heterogeneity through spatial transcriptomics, single-cell profiling, and copy number variations. *PLoS One* 2025;20:e0317115.
50. Zhou M, Zheng M, Zhou X, Tian S, Yang X, Ning Y, et al. The roles of connexins and gap junctions in the progression of cancer. *Cell Commun Signal* 2023;21:8.
51. Zhang Y, Zhang Y, Zhou S, Rehman MU, Lin F, Zhang J, et al. HTR1D regulates the PI3K/Akt signaling pathway to impact hepatocellular carcinoma development and resistance to sorafenib. *BMC Cancer* 2025;25:185.
52. Jin X, Li H, Li B, Zhang C, He Y. Knockdown and inhibition of hydroxytryptamine receptor 1D suppress proliferation and migration of gastric cancer cells. *Biochem Biophys Res Commun* 2022;620:143–9.
53. Sui H, Xu H, Ji Q, Liu X, Zhou L, Song H, et al. 5-hydroxytryptamine receptor (5-HT 1D R) promotes colorectal cancer metastasis by regulating Axin1/ β -catenin/MMP-7 signaling pathway. *Oncotarget* 2015;6:25975–87.
54. Ceyhan GO, Demir IE, Rauch U, Bergmann F, Müller MW, Büchler MW, et al. Pancreatic neuropathy results in “neural remodeling” and altered pancreatic innervation in chronic pancreatitis and pancreatic cancer. *Am J Gastroenterol* 2009;104:2555–65.
55. Geng ZH, Zhu Y, Li QL, Zhao C, Zhou PH. Enteric nervous system: the bridge between the gut microbiota and neurological disorders. *Front Aging Neurosci* 2022;14:810483.
56. Liu Y, Suzuki YJ, Day RM, Fanburg BL. Rho kinase-induced nuclear translocation of ERK1/ERK2 in smooth muscle cell mitogenesis caused by serotonin. *Circ Res* 2004;95:579–86.
57. Sei Y, Feng J, Zhao X, Forbes J, Tang D, Nagashima K, et al. Polyclonal crypt genesis and development of familial small intestinal neuroendocrine tumors. *Gastroenterology* 2016;151:140–51.