UNIVERSITY OF
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Summary of
Doctoral Thesis

Helvijs Niedra

RNA MOLECULAR FACTORS IN PITUITARY AND PANCREATIC NEUROENDOCRINE TUMORS

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MEDICINE AND HEALTH SCIENCE

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SUMMARY OF DOCTORAL THESIS

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Supervisor: Dr. biol. **Vita Rovīte**

Reviewers:

1) Professor, PhD, **Justo P. Castaño**, Maimonides Biomedical Research Institute of Cordoba (IMIBIC)
2) Professor, PhD, **Aija Linē**, Latvian Biomedical Research and Study Centre, University of Latvia
3) Dr. **Vadims Parfejevs** (PhD), University of Latvia

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ABSTRACT

Pituitary (PitNET) and pancreatic (PanNET) neuroendocrine tumors originate from anterior pituitary and pancreatic islet neuroendocrine cells, respectively, and present diagnostic and treatment challenges. PitNETs are difficult to diagnose due to their small size and location, necessitating new minimally invasive biomarkers. PanNETs are often diagnosed late, with limited understanding of stromal cell roles in their development. This thesis aimed to molecularly characterize these tumors using next-generation sequencing of circulating miRNAs in PitNET patients and spatial transcriptomics to profile PanNET tumor and stromal gene expression.

In PitNETs, plasma sequencing from inferior petrosal sinuses identified miR-7-5p and miR-375-3p as potential markers for adrenocorticotropic hormone-secreting tumors, with miR-7-5p validated as the most promising. In growth hormone-secreting tumors, plasma levels of several miRNAs, including miR-625-5p, decreased during somatostatin analog therapy, suggesting their use in monitoring treatment response. Spatial transcriptomics of PanNETs showed α-SMA-expressing stromal cells are enriched in cancer-associated fibroblast markers. Furthermore, the gene expression changes associated with tumor grade were observed in both tumor and stromal cells. In analysis of G3 PanNET it was also observed that tumor cells from regions with rich α-SMA-expressing cell presence overexpress *MMP9* gene. Lastly, in comparison against islet cells, PanNET tumor cells showed dysregulation in known oncogenic signaling pathways, mainly Phosphoinositide 3-kinase (PI3K) and MAPK signaling pathways, with consistent downregulation of tumor suppressors like early Early Growth Response Factor 1 (*EGR1*) and RAS P21 Protein Activator 4 (*RASA4*) across all three grades.

Keywords:

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ABBREVIATIONS

ACTH – adrenocorticotropic hormone
AOI – area of illumination
BIPSS – bilateral inferior petrosal sinus sampling
CAF – cancer associated fibroblast
ECM – extracellular matrix
EGR1 – Early Growth Response Factor 1
EV – extracellular vesicle
G1 – grade 1
G2 – grade 2
G3 – grade 3
GH – growth hormone
IPS – inferior petrosal sinus
MAPK – mitogen activated protein kinase
miRNA – micro ribonucleic acid
NE – neuroendocrine
NET – neuroendocrine tumor
NF-PitNET – non-functioning pituitary neuroendocrine tumor
NGS – next generation sequencing
PanNET – pancreatic neuronendocrine tumor
PDAC – pancreatic ductal adenocarcinoma
PDGF – platelet derived growth factor
PDGFR – platelet derived growth factor receptor
PitNET – pituitary neuroendocrine tumor
qPCR – quantitative polymerase chain reaction
RASA4 - RAS P21 Protein Activator 4
ROI – region of interest
SYP - synaptophysin
TGF-β – transforming growth factor beta
TME – tumor microenviroment
α-SMA – alpha smooth muscle actin

INTRODUCTION

Neuroendocrine tumors (NETs) are a diverse group of neoplasms with significant clinical and molecular heterogeneity. This variability is especially pronounced between pituitary NETs (PitNETs) and pancreatic NETs (PanNETs), which are classified differently and present distinct clinical challenges. While PanNETs are often slow growing but can metastasize, PitNETs rarely metastasize but cause significant morbidity due to hormone secretion and mass effects. Current diagnostic methods do not adequately consider the use of circulating nucleic acids and the contribution off tumor microenvironment (TME) in tumor development. Two aspects which have a potential to greatly improve the patient care; and yet, they remain understudied in NETs.

This thesis addresses two major challenges in NET research: the lack of novel for minimally invasive biomarkers that could improve PitNET diagnosis and treatment response monitoring, and a lack of information regarding molecular mechanisms that take place in the TME of NETs.

Accordingly, this thesis aims to:

- Identify circulating microRNAs (miRNAs) that with a potential to serve as blood-based biomarkers for PitNETs.
- Investigate genes involved in PanNET tumorigenesis and tumor-stroma interactions using spatial transcriptomics approaches.

To achieve these aims the following tasks were set:

- Analyze extracellular vesicle-associated miRNAs in PitNET patient plasma using small non-coding RNA sequencing.
- Validate candidate miRNAs in independent cohorts via quantitative polymerase chain reaction (qPCR).
- Use spatial transcriptomics to profile gene expression in tumor and stromal cells.
- Perform pathway enrichment analyses to elucidate dysregulated pathways and tumor-stroma crosstalk mechanisms.

THEORETICAL BASIS

1.1 The neuroendocrine system: structure and function

The neuroendocrine system is a complex regulatory network that maintains homeostasis, development, and stress responses in vertebrates (Molitch 2017). At its core are the hypothalamus and pituitary gland, which coordinate five major axes: hypothalamic-pituitary-thyroid, -adrenal, -gonadal, -somatotropic, and -mammary (White and Porterfield 2013). The hypothalamus links the nervous and endocrine systems, releasing hormones into the hypophyseal portal system to regulate the anterior pituitary (Yin and Gore 2010). These tropic hormones stimulate peripheral endocrine glands, while feedback loops maintain hormonal balance (White and Porterfield 2013).

1.2 Neuroendocrine cells: definition and classification

Neuroendocrine (NE) cells are specialized epithelial or non-epithelial cells with both endocrine and neuronal features, such as hormone secretion, excitability, and the presence of large dense core vesicles (Klöppel 2017; Oronsky et al. 2017). Key markers include chromogranin A, synaptophysin (SYP), neural cell adhesion molecule, and neuron-specific enolase (Klöppel 2017). NE cells are classified by origin: epithelial NE cells arise from the endoderm, while non-epithelial NE cells are neuroectodermal (Asa et al. 2021). Both types can form major endocrine organs or clusters, such as the anterior pituitary, parathyroids, pancreatic islets (Klöppel 2017).

1.3 Neuroendocrine tumors

NETs are a heterogeneous group of neoplasms arising from NE cells throughout the body, most commonly in the gastrointestinal tract, pancreas, lungs, and pituitary (Reccia et al. 2023). They display diverse clinical behaviors, ranging from benign to highly aggressive, and are classified based on site, hormone production, and proliferative index (Dong et al. 2024; Cives and Strosberg 2018). According to the World Health Organization classification system, classical “carcinoid” NETs are stratified into grades based measurements of either mitotic count and/or antigen Kiel-67 (Ki-67) index, with higher grades indicating more proliferative capacity, and as a result more aggressive disease (Rindi et al. 2022).

1.4 Pituitary neuroendocrine tumors

PitNETs, formerly known as pituitary adenomas, are typically benign but can cause significant morbidity through hormone hypersecretion or mass effects (Asa et al. 2022). Unlike classical "carcinoid" NETs, they are classified by hormone and pituitary cell lineage-specific transcription factor expression (Asa et al. 2022). The specific hormones assayed are growth hormone (GH), prolactin, beta subunits of thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, adrenocorticotropic hormone (ACTH), and alpha subunit. The assayed cell lineage transcription factors primarily are T-Box transcription factor 19 (*TBX19* also known as TPIT), Steroidogenic Factor 1 (*NR5A1*, also known as SF1), and Pituitary Transcript Factor 1 (*POU1F1*, also known as PIT1) (Asa et al. 2022). Despite the generally low metastatic potential of PitNETs, they are a challenge to diagnose due to their small size, with 50% measuring less than 1 cm, and obscured location within *sella turcica* region (Molitch 2017). Clinical presentation depends on PitNET type and hormone excess. GH excess causes acromegaly, ACTH excess causes Cushing's disease, and prolation excess causes hyperprolactinemia. PitNETs can also cause local, mass effect related symptoms, e.g., visual disturbances from *chiasma opticum* compression (Kasuki and Raverot 2020).

1.5 Pancreatic neuroendocrine tumors

PanNETs, part of the broader group of carcinoids representing gastroenteropancreatic tract, can be functional (hormone-secreting) or non-functional (silent) (Cives and Strosberg 2018). Functional PanNETs include insulinomas, gastrinomas, and others, presenting with distinct clinical syndromes. Non-functional PanNETs are often present late, when mass effects or metastases occur. They originate from islet cells of pancreas and, according to epigenetic signatures, they can be further stratified into β-cell-like tumors or α-cell-like tumors. PanNETs are graded by mitotic count and Ki-67 index, with higher grades correlating with worse prognosis (Dong et al. 2024). Unlike PitNETs, PanNETs have a significant risk of metastasis, particularly to the liver. Current molecular markers of PanNETs include Aristaless Related Homeobox (*ARX*) and Pancreatic and Duodenal Homeobox 1 (*PDX1*) transcription factors, which can differentiate between α-cell-like and β-cell-like tumors, and ATRX Chromatin Remodeler (*ATRX*) and Death Domain Associated Protein (*DAXX*), which are mutated in up to 60% of tumors and are known

to promote alternative lengthening of telomeres, a feature associated with worse clinical outcomes (Hong et al. 2020; Hackeng et al. 2022).

1.6 Micro RNAs in PitNET pathogenesis

miRNAs are ~22 nucleotides long non-coding RNAs that bind mainly to the 3' untranslated regions of target messenger RNAs to suppress translation (Rani and Sengar 2022). They are produced via canonical and non-canonical pathways involving processing by Drosha, DICER, or AGO2 enzymes (Kim, Lee, and Kim 2025). The mature miRNA forms part of the miRNA-induced silencing complex (miRISC), which represses translation and promotes messenger RNA degradation through recruitment of deadenylation and decapping complexes (Ambros 2024; Kuzuoğlu-Öztürk et al. 2016).

After the discovery of microRNAs (miRNAs) as key regulators of gene expression, their involvement in cancer became evident, and the first studies by Calin *et al.* and Cimmuno *et al.* showed that miR-15/16 deletions are linked to chronic lymphocytic leukemia (Calin et al. 2002; Cimmino et al. 2005) Later on, the impact of miR-15/16 in tumorigenesis was also confirmed in PitNETs (Bottoni et al. 2005). Nowdays, the advances in sequencing have expanded the catalog of human miRNAs and accelerated research into their roles of PitNETs development (Kozomara, Birgaoanu, and Griffiths-Jones 2019; Peculis, Niedra, and Rovite 2021). Recent studies have identified several miRNAs associated with PitNET development, hormone secretion, and treatment response. For example, miR-34a and miR-145 are linked to AIP-mutant PitNET aggressiveness and drug resistance, while miR-185 and miR-93 are implicated to play a role in developing resistance to somatostatin analogues and dopamine agonists, respectively (Bogner et al. 2020; Fan et al. 2015; Z. Wu et al. 2018). These findings highlight the potential of miRNAs as biomarkers and therapeutic targets in PitNETs.

1.7 Circulating microRNAs in PitNETs

Circulating nucleic acids were first detected in human blood in 1948 (MANDEL and METAIS 1948). These circulating miRNAs have since gained attention as promising liquid biopsy biomarkers due to their stability in bodily fluids and their ability to reflect disease states such as cancer and autoimmune disorders (Pozniak, Shcharbin, and Bryszewska 2022; Ciechomska et al. 2023). Unlike circulating tumor DNA, miRNAs are protected from degradation by packaging within extracellular vesicles (EVs) or ribonucleoprotein complexes, contributing to their longer half-

life and diagnostic potential (C. Wang and Liu 2022). Studies suggest EV-associated miRNAs may offer superior diagnostic value, as the genes regulated by them are enriched in oncogenic signaling pathways (Petracci et al. 2024).

In pituitary neuroendocrine tumors (PitNETs), liquid biopsy is particularly valuable due to the gland's inaccessible location and the limitations of tissue biopsies (Gossing, Frohme, and Radke 2020). However, the research remains limited on circulating miRNAs in these tumors. Current studies have attempted to identify differentially expressed of circulating miRNAs in PitNET patients under several conditions. For example, Németh *et al*. employed next generation sequencing (NGS) to analyze plasma levels of EV-derived miRNAs pre- and post-surgery. As a result they identified miR-143-3p to be significantly downregulated after resection in patients harboring non-functioning PitNETs of gonadotroph origin, suggesting its potential as a monitoring biomarker (Németh et al. 2019). Additionally, Valassi *et al*. linked the expression of two circulating (103-a-3p and miR-660-5p) miRNAs to bone tissue complications in patients with acromegaly, indicating miRNA utility in assessing both tumor status and disease-related phenotypes (Valassi et al. 2019). These findings highlight the promise of circulating miRNAs as minimally invasive tools to improve diagnostic and prognostic aspects of PitNETs.

1.8 Tumor microenvironment

Tumor is a complex ecosystem where the tumor cells are not alone, rather they are a component of the so called TME. In TME another particularly crucial component is the tumor stroma. It is a component that can promote tumor development and progression (de Visser and Joyce 2023). The stroma consists of extracellular matrix (ECM), vascular structures, and cancer-associated fibroblasts (CAFs), which interact dynamically with tumor cells (Xu et al. 2022). The tumor-stroma ratio can influence prognosis differently depending on tumor type; for pancreatic neuroendocrine tumors (PanNETs), a higher stromal proportion correlates with increased invasiveness and metastasis (Ye et al. 2024).

CAFs, primarily derived from activated fibroblasts influenced by tumor-secreted growth factors such as transforming growth factor beta (TGF-β) and platelet derived growth factors (PDGFs). Other origins of CAFs include endothelial, mesenchymal stem cells and pancreatic stellate (unique pancreatic ductal adenocarcinomas) (Glabman, Choyke, and Sato

2022; Ping et al. 2021). These cells can be regarded as the fundamental component of tumor stroma and they can be identified via expression of markers like α-SMA, FAP, and PDGFRs. Their presence in tumor stroma is often associated with therapy resistance and more aggressive phenotypes, though, due to vast heterogeneity of CAFs, some subtypes may restrain tumor growth, for example, antigen-presenting (apCAFs) can activate infiltrating T cells promoting anti-tumor immunity (Mhawech-Fauceglia et al. 2015; Vathiotis et al. 2021).

Other functionally distinct CAF subtypes that participate tumor-stroma crosstalk are myofibroblast-like (myCAFs) and inflammatory (iCAFs). These are the subtypes are often associated with worse clinical outcomes by inducing a desmoplastic environment via excessive ECM protein deposition, by suppression of adaptive immune response, and by secretion of cytokines and growth factors which are intercepted by tumor cells (Baiyao Wang et al. 2023; Zhang et al. 2023). In PanNETs, α-SMA-expressing CAFs are known to promote tumor aggressiveness and metastasis by inducing epithelial-mesenchymal transition in tumor cells, as demonstrated by co-culture and mouse model studies (Lai et al. 2024; Ye et al. 2024). These findings underscore the pivotal role of CAFs in PanNET tumor progression and highlight a need for identifying potential therapeutic targets within the TME.

MATERIALS AND METHODS

2.1 Patient recruitment and ethical approvals

Patients with PitNETs and PanNETs were recruited through the Genome Database of the Latvian Population (Rovite et al. 2018) in collaboration with major Latvian hospitals, including Pauls Stradins Clinical University Hospital and Riga East Clinical University Hospital. Additionally, PanNET samples were also obtained in collaboration with Ramón y Cajal University Hospital (Madrid, Spain). Recruitment of PitNET samples was conducted under the European Regional Development Fund (ERDF) project "RNA Molecular Determinants in the Development of Pituitary Adenoma" (project No. 1.1.1.1/18/A/089). Recruitment of PanNET patients was conducted under the ERDF project "Establishing an Algorithm for the Early Diagnosis and Follow-up of Patients with Pancreatic Neuroendocrine Tumors" (project No. 1.1.1.5/ERANET/20/03). All participants provided dual informed consent (project-specific and biobank-specific), and all procedures were approved by relevant ethical committees in Latvia and Spain, adhering to the standards set by Declaration of Helsinki. The recruitment and use of PitNET patients' samples was approved by Central Medical Ethics Committee of Latvia (protocol No.: 22.03.07/A7 and 2/18-02-21). The recruitment and use of PanNET patients' samples was approved by Central Medical Ethics Committee of Latvia (approval protocol No.: 1.1–2/67) and the Ramón y Cajal Ethical and Scientific Committees (protocol No.: 196-19).

2.2 Design of Study No. 1

NGS to profile miRNAs in plasma samples collected during bilateral inferior petrosal sinus sampling (BIPSS) from a 24-year-old female with suspected Cushing's disease. Blood was sampled from both left and right inferior petrosal sinuses via femoral vein catheters at three time periods following peripheral administration of corticotropin releasing hormone. Following the blood sample collection and plasma separation an extraction was performed to isolate circulating EV-associated miRNAs. This was followed by small non-coding RNA library preparation and NGS analysis to assess miRNA expression profiles in blood plasma from inferior petrosal sinuses. Accordingly, identified candidates were then selected for further validation by qPCR. The validation cohort included peripheral blood plasma samples from 26 PitNET patients, divided into: five patients with confirmed ACTH-secreting PitNETs and with available

pre- and post-surgical plasma samples and tumor tissues; 12 ACTH-secreting PitNETs with pre-surgical plasma samples; nine NF-PitNETs with pre surgical plasma samples.

2.3 Design of Study No. 2

This study employed NGS to compare circulating miRNA profiles in pre- and post-operative plasma from PitNET patients. The initial cohort included following groups: eight GH-secreting, 28 NF-PitNETs, four ACTH-secreting, and six prolactin-secreting PitNETs. Samples meeting quality criteria underwent small RNA-seq library preparation and NGS analysis. Differential expression analyses were used to compare miRNA expression profiles of post- and pre-operative plasma samples within each tumor group. After this, candidate miRNAs were selected for downstream qPCR validation. The selection was based on overlap between the results of differential expression analysis and literature findings. The downstream miRNA expression analyses using qPCR involved a sequential evaluation of six GH-secreting PitNET patients under somatostatin analogue treatment, followed by comparison of an independent group of 15 GH-secreting PitNETs against five NF-PitNETs, and 13 healthy controls.

2.4 Design of Study No. 3

This study focused on spatial transcriptomic profiling of tumor α-SMA-expressing stromal cells in PanNETs. The cohort consisted of eight untreated PanNET patients (three grade 1 [G1], four grade 2 [G2], one grade 3 [G3]). Formalin-fixed paraffin-embedded (FFPE) tissue blocks were analyzed using the GeoMx® Digital Spatial Profiler (Nanostring). Regions of interest (ROIs) were selected using fluorescent antibodies for SYP (tumor cells) and α-SMA (stromal cells), with SYTO83 used for nuclear staining. The signal from these markers was also used to guide area of illumination (AOI) segmentation – positions in the tissue from which the GeoMx® Cancer Transcriptome Atlas (Nanostring) panel probe barcodes are collected. In six patients, adjacent non-tumor pancreatic tissue was also profiled which included gene expression assessment in acinar and islet compartments separately.

2.5 PitNET patient blood sample processing

Blood samples, whether from peripheral veins or the inferior petrosal sinuses IPSs, were collected in EDTA tubes, kept at room temperature, and centrifuged twice (2000 RPM and 4000 RPM,

10 minutes each at 25°C) to separate plasma. Plasma was aliquoted (1 mL) and stored at -80°C until further analysis.

2.6 Isolation of circulating EV-associated miRNAs from plasma

For both studies (No. 1 and No. 2), the focus was on extracellular vesicle (EV)-associated plasma miRNAs. Total RNA, including miRNAs, was extracted using the exoRNeasy Midi Kit (Qiagen), which involves isolation of EVs and subsequent miRNA purification. To monitor for hemolysis during blood collection, samples were tested for red blood cell miRNA contamination. For the BIPSS patient, 52 synthetic RNA spike-ins from QIAseq miRNA Library QC Spike-ins kit (Qiagen) were added to all plasma samples for additional quality control.

2.7 miRNA library preparation for NGS

Small RNA libraries were prepared using the Small RNA-Seq Library Prep Kit (Lexogen). Size selection was performed using BluePippin electrophoresis (Sage Science) to enrich for Illumina adapter ligated miRNA fragments (target fragment size 144–153 bp). Final library quality was assessed using the 2100 Bioanalyzer (Agilent) and Qubit 2.0 fluorometer (Thermo Fisher). Libraries meeting quality criteria were sequenced on MiSeq and NextSeq 500 platforms (Illumina), targeting 3–5 million reads per sample.

2.8 qPCR analysis

For validation and hemolysis quality control, SYBR Green-based qPCR was performed using miRCURY LNA miRNA PCR Assay kits (Qiagen). cDNA was synthesized with the miRCURY LNA RT Kit (Qiagen), and qPCR was performed on the ViiA 7 System (Thermo Fisher). Hemolysis was assessed by comparing ΔCt values for miR-23a and miR-451; samples with ΔCt >5 were excluded from NGS analysis due to potential red blood cell miRNA contamination.

2.9 PanNET sample preparation for spatial transcriptomics

Formalin fixed, paraffin embedded PanNET tissue sections (4–6 μm) underwent antigen retrieval and overnight hybridization with the GeoMx® Cancer Transcriptome Atlas panel (Nanostring). Slides were blocked and stained with fluorescent antibodies for α-SMA and SYP, and nuclei were visualized with Syto83. Following GeoMx® Digital Spatial Profiler instrument (Nanostring) guided oligo tag release, PCR was performed with released barcodes using overhang Illumina

index primers. Final library quality was checked using the 2100 Bioanalyzer (Agilent) and Qubit 3.0 (Thermo Fisher), and sequencing was performed on the NovaSeq 6000 system (Illumina).

2.10 Small RNA sequencing data preprocessing

FASTQ files from small RNA sequencing were processed using CLC Genomics Workbench (v20.0.4). Processing included 3' adapter trimming, removal of low-quality reads (Phred score <20 or <30), and length trimming (15–55 bases). Mature miRNA quantification was performed using miRBase v22 as a reference, allowing up to two base mismatches and isomiR quantification within two bases. For the BIPSS patient, spike-in quantification was performed using the QIAseq miRNA Library QC Spike-ins dataset (available on manufactures website).

2.11 Statistical analyses for Study No. 1

To compare miRNA expression in left and right IPS plasma, data normalization was performed using the trimmed mean of M values (TMM) method. The top 50 miRNAs with highest variance and at least 1000 reads were visualized in a heatmap. Differential expression analysis was conducted using the CLC Genomics Workbench tool (Qiagen), equivalent to the edgeR package, which uses a negative binomial model suitable for small amount of biological replicates as in case of BIPSS patient plasma comparison. For qPCR data, the Livak's ΔΔCt method was used. Additional statistical analyses were performed in R (v4.1.2) using paired t-tests or Wilcoxon rank sum tests, depending on normality (assessed by the Shapiro-Wilk test).

2.12 Statistical analyses for Study No. 2

Non-normalized miRNA count matrices from pre- and post-operative plasma samples were analyzed using DESeq2 (v1.30.1) in R (v4.0.3) (Love, Huber, and Anders 2014). Principal component analysis (PCA) with variance-stabilizing transformed data was used to estimate batch effects. The DESeq2 model included sampling time, tumor type, and sequencing batch as variables. Differentially expressed miRNAs and $Log_2FoldChange$ values were identified using DESeq2 Wald test, and results were corrected for multiple testing using False Discovery Rate (FDR) method.

2.13 Data processing and statistical analyses for Study No. 3

For spatial transcriptomics, raw sequencing data were processed according to the GeoMx DSP analysis pipeline developed by Nanostring. Gene expression was estimated by quantifying sequenced Cancer Transcriptome Atlas panel probes for each defined AOI. Data normalization and differential expression analyses were performed using the Nanostring GeoMx DSP analysis suite software (Nanostring). Cell type deconvolution analysis and reverse deconvolution analysis was performed using SpatialDecon package (v1.8.0) in R (v4.2.2) (Danaher et al. 2022). Further pathway enrichment analyses with differentially expressed genes were performed using STRING database (v11.5) and visualization of results was performed using R (v4.2.2) and Cytoscape (v3.9.1) (Szklarczyk et al. 2023).

RESULTS AND DISCUSSION

NETs are molecularly and clinically diverse, presenting significant challenges in healthcare, particularly due to the lack of novel molecular biomarkers for minimally invasive testing (Sultana et al. 2023). The TME, which plays a critical role in other cancers such as pancreatic ductal adenocarcinoma (PDAC), gastric, and breast cancers, remains underexplored in NET development (de Visser and Joyce 2023). PitNETs, although mostly benign with metastatic cases being rare (0.2%) (Iglesias 2023), still significantly impact patient quality of life and mortality through hormone hypersecretion and tumor mass effects (Toma et al. 2025). Their anatomical location complicates diagnosis, highlighting the urgent need for minimally invasive biomarkers with diagnostic and prognostic utility (Butz 2022). In pancreatic NETs (PanNETs), molecular markers such as *ATRX*/*DAXX* have been linked to increased tumor aggressiveness (Hackeng et al. 2022). However, the role of stromal cells and the TME, particularly in fibrotic tumors and it's associated with tumor agressiveness, remains to be studied further (Chatterjee et al. 2020; Lai et al. 2024).

This dissertation aims to address these gaps in NET research using NGS-based analyses to identify potential RNA markers that could be used to improve patient diagnostics and prognostics. Accordingly, several significant observations were made. In the first two studies on circulating miRNA profiling in PitNET patients, potential miRNAs were identified that can distinguish ACTH-secreting and GH-secreting PitNETs. The third study on spatial RNA profiling of PanNET tissues revealed key genes expressed by α-SMA-positive stromal cells, among which were PDGFR and NOTCH pathway-related genes, potentially mediating tumor-stroma communication. Additionally, it was observed that G3 tumor cells located near α-SMA-positive cells overexpress *MMP9*. Lastly, this study also demonstrated that transcriptional changes can be observed in both tumor and α-SMA-expressing stromal cells as tumor grades progress and pinpointed the genes dysregulated in tumor cells compared to non-tumor neuroendocrine counterparts.

3.1 Circulating miRNAs in PitNETs

The exploration of circulating miRNAs as minimally invasive biomarkers for PitNETs is still in early stages, largely due to the typically benign nature and small size of these tumors which questions the feasibility of detecting PitNET derived circulating nucleic acids (Butz

2022). However, prior work by our research group demonstrated that somatic mutations found in PitNET tissues can also be detected in circulating cell-free DNA (Megnis et al. 2019), confirming the possibility to detect PitNET derived cell free DNA. Accordingly, the first two studies in this thesis aimed to expand on study of circulating nucleic acids in PitNETs by identifying potential circulating miRNAs that could serve as diagnostic and monitoring tools for functioning PitNETs, specifically ACTH- and GH-secreting.

In Study No. 1, NGS was performed on plasma samples collected during BIPSS the current gold standard procedure for diagnosing ACTH-secreting PitNETs (Zampetti et al. 2016). By comparing miRNA profiles from left vs. right IPS it was revealed that there was a significant increase in several miRNAs in the left IPS after stimulation by peripherally administered corticotropin-releasing hormone. Amongst the upregulated miRNAs there were two candidates of interest: miR-375-3p and miR-7-5p. Both are known to be highly expressed in pituitary tissue; and in functional studies they have been implicated to play are role in hormone exocytosis pathways (Gümürdü et al. 2017; Zacharjasz et al. 2024). Furthermore, qPCR-based tests on miR-375-3p and miR-7-5p in peripheral blood revealed that miR-7-5p levels significantly decreased after tumor removal (Figure 1, panels A-C). Additionally, miR-7-5p showed a stable expression in ACTH-secreting PitNET tissues (Figure 1D) and its levels were significantly higher in plasma of ACTH-secreting PitNET patients versus plasma from patients with NF-PitNETs (Figure 1E). These findings highlight the potential of miR-7-5p as a novel, minimally invasive biomarker for diagnosis and treatment monitoring of ACTH secreting PitNETs.

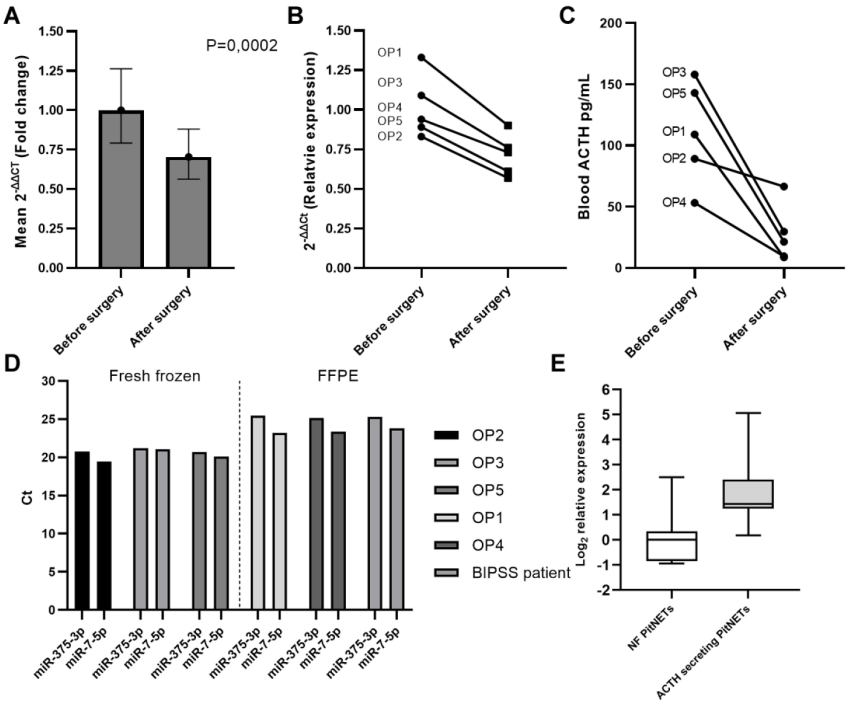

Figure 1. qPCR-based validation of candidate miRNAs. Figures A–C represent the analysis of miR-7-5p in the plasma of five ACTH-secreting PitNET patients before and after tumor surgery. A - panel shows the expression values, which were calculated using the ΔΔCt method and are displayed as mean fold change between groups. In our sample set of five patients, miR-7-5p was slightly downregulated in plasma 24 hours after surgery (P = 0.0002). B - panel also represents the relative expression of miR-7-5p before and after surgery, but for each patient individually. The P value for the comparison was calculated using a paired t-test with ΔCt values. C - graph shows ACTH measurements taken before and one month after PitNET surgery. D – this panel confirms the expression of miR-375-3p and miR-7-5p in tumor tissues, including the BIPSS patient. E – panel shows the difference in expression between non-functioning (NF) PitNETs and ACTH-secreting PitNETs, clearly indicating that miR-7-5p is overexpressed in ACTH-secreting PitNETs compared to NF-PitNETs. Figures were directly adopted from Study No. 1 with minor changes in Figure C for visual clarity.

The study No. 2 expanded further on circulating miRNAs in plasma of ACTH secreting PitNET patients by profiling circulating miRNAs in pre- and post-operative plasma samples from a cohort of four ACTH-secreting PitNET patients. Among the miRNAs analyzed, miR-141-3p was significantly upregulated following surgery. However, the biological significance of this increase remains unclear, as previous studies have produced inconsistent results regarding the role of miR-141-3p in PitNETs (Belaya et al. 2020; Enguita et al. 2023; Vetrivel et al. 2021). The post-operative elevation is likely attributable to tumor cell undergoing necrosis (Pozniak, Shcharbin, and Bryszewska 2022), rather than active secretion, limiting its usefulness as a biomarker to detect ACTH secreting PitNETs.

In parallel, the study No 2. also carried out an NGS-based investigation of GH-secreting PitNETs and identified a multitude of differentially expressed miRNAs of which seven circulating miRNAs where chosen as candidates for further testing by qPCR. In the tests of these seven candidates, four miRNAs (miR-625-5p, miR-181a-2-3p, miR-503-3p, and miR-130b-3p) showed significant downregulation during the first month of somatostatin analog treatment. In following months only miR-625-5p remained downregulated at three months. This persistent downregulation may be associated with changes in the insulin like growth factor 1 (IGF-1) receptor signaling pathway, as miR-625-5p is known to regulate the expression of IGF-1 receptor (L. Wu et al. 2022; Al-Samerria and Radovick 2021). However, this needs to be further validated via functional studies. Additionally, miR-625-5p was found to be downregulated in GH-secreting PitNET plasma compared to non-functioning tumors and healthy controls, providing another suggestion that it may play a role in tumor pathogenesis and could serve as marker to distinguish GH secreting tumors (He et al. 2019).

Taking together, these studies demonstrate the feasibility and potential of circulating miRNAs as non-invasive biomarkers for PitNETs. The identified association of miR-7-5p and miR-625-5p with ACTH- and GH-secreting tumors, provides novel markers for development of blood-based circulating miRNA assays to improve diagnosis, patient stratification, and monitoring of treatment response. Further validation in larger cohorts and functional studies are warranted to fully understand their applicability as diagnostic tools and to elucidate their underlying roles in PitNET pathogenesis.

3.1 Spatial RNA profiling of PanNETs

Recent advances in cancer research have highlighted the crucial role of the TME in modulating tumor behavior. The TME comprises a dynamic network of tumor and host cells, interacting through direct cell-to-cell contacts (integrins, cadherins, selectins, immunoglobulins) and via paracrine signals (cytokines, growth factors) (de Visser and Joyce 2023). Spatial profiling technologies have now further helped to advance the exploration of TME at a more rapid pace (Elhanani, Ben-Uri, and Keren 2023). Despite this, the TME in neuroendocrine neoplasms, including PanNETs, remains relatively underexplored, with most knowledge derived from immunohistochemistry and *in vitro* co-culture studies (Takahashi et al. 2018; Cuny et al. 2022; Z. Chen et al. 2024; Amin et al. 2023). For this reason, the third study (Study No. 3) of this thesis aimed to elucidate tumor-stroma interactions in PanNETs by performing spatial RNA profiling on eight PanNET samples (representing all three grades) using Nanostring GeoMx® technology (Figure 2A). α-SMA (alpha-smooth muscle actin) was selected as the primary marker for stromal cell visualization due to its established association with poor prognosis in various cancers and its expression by activated fibroblasts (CAFs), particularly the myCAF subtype (Zhao et al. 2023; Sculthorpe et al. 2025; Muchlińska et al. 2022; Vathiotis et al. 2021).

The assessment of gene expression in each AOI was followed by cell type deconvolution analysis (Figure 2B), which revealed a significant enrichment of stromal cells in α-SMA-expressing AOIs, especially in the G3 tumor sample. This aligns with findings by Ye *et al*., who observed that higher stromal content correlates with increased tumor grade and worse prognosis (Ye et al. 2024). In G1 and G2 tumors, α-SMA AOIs were still dominated by tumor cells, with notable infiltration by immune cells (T cells and myeloid cells). Since the GeoMx analysis heavily relies on morphology markers, this perhaps suggests that α-SMA alone is insufficient for exclusive CAF identification and combining it with additional CAF markers such as FAP and PDGFR may improve the specificity of identifying these particular cell types.

Despite the presence of non-stromal cells in α-SMA AOIs, CAF-related gene signatures were still evident. Across all tumor grades, α-SMA AOIs showed enrichment of collagen family genes (COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL6A3), and other CAF related secretome proteins, namely fibronectin (*FN1*), and TGF-β (*TGFB1*). Both fibronectin and TGF-β can be secreted by both tumor cells and CAFs, and are known known to promote further CAF

differentiation (Yoon et al. 2021). Furthermore, pathway analysis of genes upregulated in α-SMA AOIs revealed upregulation of extracellular matrix (ECM) remodeling pathways, MET signaling, PDGF signaling, and immune system related signaling (Figure 2, panels C-D). There is also evidence of NOTCH signaling, despite it not being present in the list of top 15 overrepresented pathways, as across all tumor grades α-SMA cells overexpressed *NOTCH3* receptor. There was also a significant difference in gene expression of α-SMA-expressing cell AOIs between different tumor grades. However, since there can be many types of stromal cells, including various CAF subtypes, it would be interesting to conduct a more expansive study to understand whether this difference in gene expression between tumor grades is linked to changes in a one specific CAF subtype or it reflects overall shift in subtype populations. In such a study the identification of additional CAF subtypes could be performed by inclusion of additional markers: interleukin 6 (IL-6) and PDGFRA for inflammatory CAFs, α-SMA and PDGFRB for myCAFs, and CD74 for apCAFs (Zhang et al. 2023).

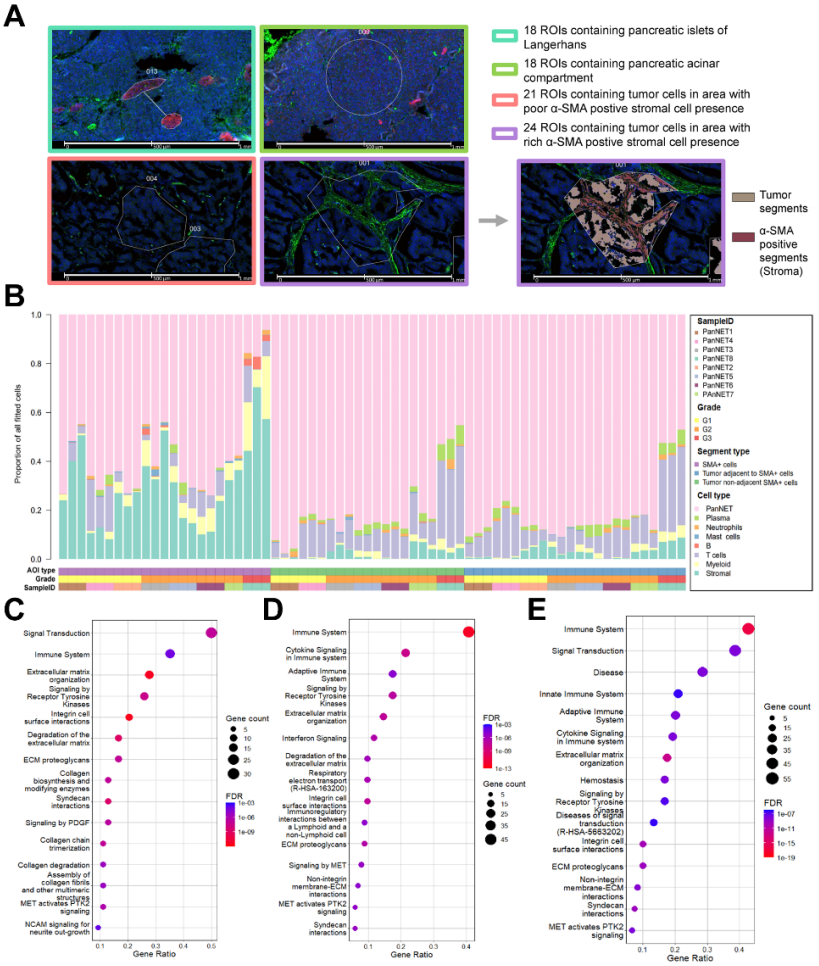

Figure 2. Spatial profiling of RNA in PanNETs. Figure A – study design. ROI – region of interest, AOI – area of illumination. To profile RNA in tumor tissues two kinds of ROIs were selected, ROI with tumor cells but with poor presence of adjacent α-SMA-expressing cells, and ROI with tumor cells and rich abundance of adjacent α-SMA-cells. Figure B – results of cell deconvolution analysis showed relative abundances of cell types in each of the analyzed AOI. Figures C, D, and E – results of overrepresentation analysis using with genes upregulated/downregulated in α-SMA-expressing cells specifically. The graphs represent only the top 15 pathways according to FDR value. Here it can be observed that amongst the top 15 enriched pathways are pathways related to extracellular matrix organization, immune interactions, signaling by PDGF and MET signaling. Pathway database – Reactome. Analysis itself was done using STRING v11.5 website application. C – analysis in G1 tumors; D – analysis in G2 tumors, E – analysis in G3 tumor.

Recalling the fact that CAFs are known to drive tumor progression through direct and paracrine interactions (Maneshi et al. 2021), for example, in PDAC, CAFs crosstalk with tumor cells induce EMT pathways, promoting aggressive tumor phenotypes (Guinn et al. 2025); raises a question whether similar effect of CAFs can be observed in PanNETs. In order to investigate this, tumor AOIs from regions with abundant α-SMA-expressing stromal cell presence were compared to those with poor α-SMA-expressing stromal cell presence (for reference, inspect Figure 2A). The results were somewhat contrary to expectations and prior findings (Ye et al. 2024), as no significant gene expression differences were observed in G1 and G2 tumors. This suggests that the proximity of α-SMA-expressing cells to tumor cells has a minimal impact; however, there is also a possibility that the chosen methodological approach is lacking in sensitivity to identify genes in tumor cells that would be regulated by closely residing α-SMA cells. However, in G3 tumors, *MMP9* was significantly upregulated in tumor cells from regions with abundant presence of α-SMA-expressing stromal cells. *MMP9*, a matrix metalloproteinase, facilitates tumor invasion and metastasis by remodeling the ECM and promoting EMT (Radisky and Radisky 2010; Egeblad and Werb 2002; Wiechec et al. 2021; Shan et al. 2017). Overexpression of *MMP9* is linked to poor prognosis in several cancers, and MMP9 inhibitors have been explored clinically, albeit with limited success (Shah et al. 2021). These findings warrant further functional studies of *MMP9* role in PanNETs.

Lastly, to observe genes altered in PanNET cells, tumor AOIs were compared to normal islet cell AOIs. Across all grades, dysregulated genes were enriched in Mitogen-Activated Protein Kinase (MAPK) signaling pathways. Here, Rat sarcoma virus (RAS) proteins (NRAS, KRAS, HRAS) mediate MAPK signaling, which is commonly mutated in cancers, especially PDAC (Bahar, Kim, and Kim 2023; Waters and Der 2018). While RAS mutations are rare in PanNETs (Konukiewitz et al. 2018), this study found downregulation of *RASA4* – a negative regulator of RAS activity. *RASA4* downregulation has been linked to poor survival in cervical cancer (J. Chen et al. 2021). PI3K signaling pathways were also dysregulated in PanNET cell AOIs, with consistent downregulation of *EGR1* across all PanNET grades. *EGR1*, a transcription factor, regulates fibrosis, injury response, and immune processes (Saha et al. 2021; Bin Wang et al. 2021). According to STRING database, it interacts with TP53 and MAPK3 (Szklarczyk et al. 2023) and can activate

Phosphatase and Tensin Homolog (PTEN), a tumor suppressor often downregulated in PanNETs (Chang et al. 2022; Virolle et al. 2001; Baron et al. 2006), including G2 and G3 tumors of this study. *EGR1* role in cancer is context-dependent, promoting metastasis in some cancers and suppressing it in others (Saha et al. 2021). Additional consistently downregulated genes included *CD99*, Serine Peptidase Inhibitor Kazal Type 1 (*SPINK1*), Phospholipase A2 Group IB (*PLA2G1B*), and retinol binding protein 4 (*RBP4*), all of which are pancreas-specific, according to Human Protein Atlas, and essential for normal endocrine and exocrine function (Thul and Lindskog 2018). Their downregulation likely reflects tumor dedifferentiation. Notably, loss of *CD99* has been associated with worse prognosis in PanNETs (Ali et al. 2006; Goto et al. 2004), and *RBP4* downregulation correlates with poor survival and reduced immune infiltration in liver cancer (Li et al. 2021).

CONCLUSIONS

1 Circulating miR-375-3p and miR-7-5p could serve non-invasive diagnostic markers, potentially providing an alternative to the highly invasive BIPSS procedure for diagnosing Cushing's disease.
2 The direct upregulation of circulating miR-375-3p and miR-7-5p in blood plasma following administration of CRH indicates a that these miRNAs possibly are components of ACTH secretion pathway. Further functional studies are needed to confirm this hypothesis.
3 The downregulation of miR-625-5p in plasma from GH-secreting PitNET patients after SSA treatment, along with its association with regulation of IGF-1 signaling, suggests a direct role in PitNET pathogenesis and utility as a biomarker for blood-based assay development.
4 In PanNETs, the enrichment of core extracellular matrix genes (*COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, *COL5A2*, *COL6A3*, and *FN1*) and the overrepresentation of ECM-modification, PDGFR, NOTCH and MET signaling related pathways in α-SMA-expressing stromal cells strongly support the presence of cancer-associated fibroblasts and underline their involvement in remodeling of PanNET microenvironment.
5 The spatial proximity of CAFs to tumor cells appears to have a limited impact on tumor gene expression, except in the G3 PanNET case, where MMP9 was found to be upregulated in tumor cells from regions enriched in α-SMA-expressing cells. However, this observation is based on a single case of G3 tumor.
6 As PanNETs progress to higher grades, distinct shifts in gene expression profiles are observed in both tumor cells and, to a lesser extent, in the surrounding α-SMA-expressing stromal cells.
7 The dysregulated genes in tumor cells compared to pancreatic islet cells were related to PI3K and MAPK signaling and several genes were found to be consistently downregulated across all tumor grades, amongst which were *EGR1* and *RASA4* – genes with tumor suppressive properties.

MAIN THESIS OF DEFENCE

1 miR-7-5p can be used to distinguish ACTH secreting patients from non-functioning PitNET patients and healthy control; therefore it is a valuable candidate for circulating miRNA-based assay development to diagnosed ACTH secreting PitNETs.
2 miR-625-5p could serve as valid candidate blood-based biomarkers for further testing in larger GH secreting PitNET patient cohorts.
3 The upregulated genes in α-SMA strongly indicates presence of CAFs and their crosstalk with tumor cells is likely mediated through PDGF, NOTCH, and MET signaling.
4 G3 PanNET tumor cells in close proximity to α-SMA expressing cells have an increased expression of *MMP9*.
5 As PanNET progresses in grade, changes in gene expression occur not only in tumor cells but also in α-SMA stromal cells.

LIST OF ORIGINAL PUBLICATIONS

Study No. 1 – Niedra H, Peculis R, Konrade I, Balcere I, Romanovs M, Steina L, Stukens J, Sokolovska J, Klovins J, Rovite V. Case Report: Micro-RNAs in Plasma From Bilateral Inferior Petrosal Sinus Sampling and Peripheral Blood From Corticotroph Pituitary Neuroendocrine Tumors. Front Endocrinol (Lausanne). 2022 Apr 22;13:748152. doi: 10.3389/fendo.2022.748152.

Study No. 2 – Niedra H, Peculis R, Litvina HD, Megnis K, Mandrika I, Balcere I, Romanovs M, Steina L, Stukens J, Breiksa A, Nazarovs J, Sokolovska J, Liutkeviciene R, Vilkevicute A, Konrade I, Rovite V. Genome wide analysis of circulating miRNAs in growth hormone secreting pituitary neuroendocrine tumor patients' plasma. Front Oncol. 2022 Sep 9;12:894317. doi: 10.3389/fonc.2022.894317.

Study No. 3 – Niedra H, Peculis R, Saksis R, Mandrika I, Vilisova S, Nazarovs J, Breiksa A, Gerina A, Earl J, Ruz-Caracuel I, Rosas MG, Pukitis A, Senterjakova N, Rovite V. Tumor and α-SMA-expressing stromal cells in pancreatic neuroendocrine tumors have a distinct RNA profile depending on tumor grade. Mol Oncol. 2025 Mar;19(3):659-681. doi: 10.1002/1878-0261.13727. Epub 2024 Sep 8.

APPROBATION OF RESEARCH

1 **Niedra H**, Peculis R, Breiksa A, Gerina A, Vilisova S, Earl J, Ruz-Caracuel I, Rovite V. Spatially resolved transcriptomics analysis of cancer associated fibroblasts in pancreatic neuroendocrine tumors. 2023 July 8. 47th FEBS Congress. Poster presentation.

2 **Niedra H**, Peculis R, Breiksa A, Gerina A, Vilisova S, Earl J, Ruz-Caracuel I, Rovite V. Digital Spatial Profiling of RNA in Tumor Cells and α-SMA-Positive Stromal Cells of Pancreatic Neuroendocrine Tumors. 2024 October 10. PMNET forum 2024. Oral presentation.

3 **Niedra H**, Peculis R, Litvina H D, Megnis K, Balcere I, Romanovs M, Steina L, Stukens J, Breiksa A, Nazarovs J, Sokolovska J, Liutkeviciene R, Vilkeviciute A, Konrade I, Rovite V. Genome wide analysis of circulating miRNAs in growth hormone secreting pituitary neuroendocrine tumor patients' plasma. 2022 July 15. FEBS3+ conference. Poster presentation.

4 **Niedra H**, Peculis R, Konrade I, Balcere I, Romanovs M, Steina L, Stukens J, Sokolovska J, Klovins J, Rovite V. Discovery of novel miRNA markers for pituitary neuroendocrine tumour. 2022 June 11, ESHG 2022 Congress. Poster presentation (presented by Rovite V.)

5 **Niedra H**, Peculis R, Litvina H D, Megnis K, Balcere I, Romanovs M, Steina L, Stukens J, Breiksa A, Nazarovs J, Sokolovska J, Liutkeviciene R, Vilkeviciute A, Konrade I, Rovite V. Genome wide analysis of circulating miRNAs in growth hormone secreting pituitary neuroendocrine tumor patients' plasma. 2022 February 18. Latvijas Bioķīmiķu Biedrība conference. Oral presentation.

6 **Niedra H**, Peculis R, Konrade I, Balcere I, Romanovs M, Steina L, Stukens J, Sokolovska J, Klovins J, Rovite V. Landscape of circulating miRNAs in plasma from bilateral inferior petrosal sinus sampling of corticotroph pituitary neuroendocrine tumor. 2021 March 22. RSU Research Week 2021. Oral presentation.

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REFERENCES

1. Al-Samerria, Sarmed, and Sally Radovick. 2021. “The Role of Insulin-like Growth Factor-1 (IGF-1) in the Control of Neuroendocrine Regulation of Growth.” *Cells* 10 (10). https://doi.org/10.3390/cells10102664.
2. Ali, Abdullah, Stefano Serra, Sylvia L Asa, and Runjan Chetty. 2006. “The Predictive Value of CK19 and CD99 in Pancreatic Endocrine Tumors.” *The American Journal of Surgical Pathology* 30 (12): 1588–94. https://doi.org/10.1097/01.pas.0000213309.51553.01.
3. Ambros, Victor. 2024. “MicroRNA-Mediated Gene Regulation and the Resilience of Multicellular Animals.” *Postepy Biochemii* 70 (1): 62–70. https://doi.org/10.18388/pb.2021_515.
4. Amin, Tania, Fabrice Viol, Jenny Krause, Martina Fahl, Corinna Eggers, Fayez Awwad, Benjamin Schmidt, et al. 2023. “Cancer-Associated Fibroblasts Induce Proliferation and Therapeutic Resistance to Everolimus in Neuroendocrine Tumors through STAT3 Activation.” *Neuroendocrinology* 113 (5): 501–18. https://doi.org/10.1159/000528539.
5. Asa, Sylvia L., Ozgur Mete, Michael D. Cusimano, Ian E. McCutcheon, Arie Perry, Shozo Yamada, Hiroshi Nishioka, et al. 2021. “Pituitary Neuroendocrine Tumors: A Model for Neuroendocrine Tumor Classification.” *Modern Pathology* 34 (9): 1634–50. https://doi.org/10.1038/s41379-021-00820-y.
6. Asa, Sylvia L, Ozgur Mete, Arie Perry, and Robert Y Osamura. 2022. “Overview of the 2022 WHO Classification of Pituitary Tumors.” *Endocrine Pathology* 33 (1): 6–26. https://doi.org/10.1007/s12022-022-09703-7.
7. Bahar, Md Entaz, Hyun Joon Kim, and Deok Ryong Kim. 2023. “Targeting the RAS/RAF/MAPK Pathway for Cancer Therapy: From Mechanism to Clinical Studies.” *Signal Transduction and Targeted Therapy* 8 (1): 455. https://doi.org/10.1038/s41392-023-01705-z.
8. Baron, V, E D Adamson, A Calogero, G Ragona, and D Mercola. 2006. “The Transcription Factor Egr1 Is a Direct Regulator of Multiple Tumor Suppressors Including TGFbeta1, PTEN, P53, and Fibronectin.” *Cancer Gene Therapy* 13 (2): 115–24. https://doi.org/10.1038/sj.cgt.7700896.
9. Belaya, Zhanna, Patimat Khandaeva, Larisa Nonn, Alexey Nikitin, Alexander Solodovnikov, Ivan Sitkin, Andrey Grigoriev, et al. 2020. “Circulating Plasma MicroRNA to Differentiate Cushing’s Disease From Ectopic ACTH Syndrome.” *Frontiers in Endocrinology* 11:331. https://doi.org/10.3389/fendo.2020.00331.
10. Bogner, Eva-Maria, Adrian F Daly, Sebastian Gulde, Auli Karhu, Martin Irmler, Johannes Beckers, Hermine Mohr, Albert Beckers, and Natalia S Pellegata. 2020. “MiR-34a Is Upregulated in AIP-Mutated Somatotropinomas and Promotes Octreotide Resistance.” *International Journal of Cancer* 147 (12): 3523–38. https://doi.org/https://doi.org/10.1002/ijc.33268.

11. Bottoni, Arianna, Daniela Piccin, Federico Tagliati, Andrea Luchin, Maria Chiara Zatelli, and Ettore C degli Uberti. 2005. "MiR-15a and MiR-16-1 down-Regulation in Pituitary Adenomas." *Journal of Cellular Physiology* 204 (1): 280–85. https://doi.org/10.1002/jcp.20282.
12. Butz, Henriett. 2022. "Circulating Noncoding RNAs in Pituitary Neuroendocrine Tumors-Two Sides of the Same Coin." *International Journal of Molecular Sciences* 23 (9). https://doi.org/10.3390/ijms23095122.
13. Calin, George Adrian, Calin Dan Dumitru, Masayoshi Shimizu, Roberta Bichi, Simona Zupo, Evan Noch, Hansjuerg Aldler, et al. 2002. "Frequent Deletions and Down-Regulation of Micro- RNA Genes MiR15 and MiR16 at 13q14 in Chronic Lymphocytic Leukemia." *Proceedings of the National Academy of Sciences of the United States of America* 99 (24): 15524–29. https://doi.org/10.1073/pnas.242606799.
14. Chang, Tsung-Ming, Pei-Yi Chu, Hui-You Lin, Kuo-Wei Huang, Wen-Chun Hung, Yan-Shen Shan, Li-Tzong Chen, and Hui-Jen Tsai. 2022. "PTEN Regulates Invasiveness in Pancreatic Neuroendocrine Tumors through DUSP19-Mediated VEGFR3 Dephosphorylation." *Journal of Biomedical Science* 29 (1): 92. https://doi.org/10.1186/s12929-022-00875-2.
15. Chatterjee, Deyali, Nikolaos A Trikalinos, Greg A Williams, Jingxia Liu, William G Hawkins, and Chet Hammill. 2020. "Intratumoral Fibrosis and Tumor Growth Pattern as Prognostic Factors in Optimally Resected Pancreatic Neuroendocrine Neoplasms: An Analysis of 168 Cases." *Pancreas* 49 (2): 255–60. https://doi.org/10.1097/MPA.0000000000001478.
16. Chen, Junying, Jinbing Huang, Qiaoqiao Huang, Ji Li, Erling Chen, and Wensheng Xu. 2021. "RASA4 Inhibits the HIFα Signaling Pathway to Suppress Proliferation of Cervical Cancer Cells." *Bioengineered* 12 (2): 10723–33. https://doi.org/10.1080/21655979.2021.2002499.
17. Chen, Zhijiang, Shizhao Lin, Feihong Liang, Zelin Hou, Yuanyuan Yang, Heguang Huang, and Yu Pan. 2024. "The Prognostic and Therapeutic Value of the Tumor Microenvironment and Immune Checkpoints in Pancreatic Neuroendocrine Neoplasms." *Scientific Reports* 14 (1): 24669. https://doi.org/10.1038/s41598-024-75882-4.
18. Ciechomska, Marzena, Leszek Roszkowski, Tomasz Burakowski, Magdalena Massalska, Anna Felis-Giemza, and Adria-Jaume Roura. 2023. "Circulating MiRNA-19b as a Biomarker of Disease Progression and Treatment Response to Baricitinib in Rheumatoid Arthritis Patients through MiRNA Profiling of Monocytes." *Frontiers in Immunology* 14:980247. https://doi.org/10.3389/fimmu.2023.980247.
19. Cimmino, Amelia, George Adrian Calin, Muller Fabbri, Marilena V Iorio, Manuela Ferracin, Masayoshi Shimizu, Sylwia E Wojcik, et al. 2005. "MiR-15 and MiR-16 Induce Apoptosis by Targeting BCL2." *Proceedings of the National Academy of Sciences of the United States of America* 102 (39): 13944–49. https://doi.org/10.1073/pnas.0506654102.
20. Cives, Mauro, and Jonathan R Strosberg. 2018. "Gastroenteropancreatic

Neuroendocrine Tumors." *CA: A Cancer Journal for Clinicians* 68 (6): 471–87. https://doi.org/10.3322/caac.21493.
21. Cuny, Thomas, Peter M van Koetsveld, Grégoire Mondielli, Fadime Dogan, Wouter W de Herder, Anne Barlier, and Leo J Hofland. 2022. "Reciprocal Interactions between Fibroblast and Pancreatic Neuroendocrine Tumor Cells: Putative Impact of the Tumor Microenvironment." *Cancers* 14 (14). https://doi.org/10.3390/cancers14143481.
22. Danaher, Patrick, Youngmi Kim, Brenn Nelson, Maddy Griswold, Zhi Yang, Erin Piazza, and Joseph M Beechem. 2022. "Advances in Mixed Cell Deconvolution Enable Quantification of Cell Types in Spatial Transcriptomic Data." *Nature Communications* 13 (1): 385. https://doi.org/10.1038/s41467-022-28020-5.
23. Dong, Song Chen, Qi Yun Tang, Lu Wang, Fang Fang, Dou Sheng Bai, Sheng Jie Jin, Bao Huan Zhou, and Guo Qing Jiang. 2024. "Characteristics and Risk Differences of Different Tumor Sizes on Distant Metastases of Pancreatic Neuroendocrine Tumors: A Retrospective Study in the SEER Database." *Hepatobiliary and Pancreatic Diseases International* 24 (2): 188–96. https://doi.org/10.1016/j.hbpd.2024.07.001.
24. Egeblad, Mikala, and Zena Werb. 2002. "New Functions for the Matrix Metalloproteinases in Cancer Progression." *Nature Reviews. Cancer* 2 (3): 161–74. https://doi.org/10.1038/nrc745.
25. Elhanani, Ofer, Raz Ben-Uri, and Leeat Keren. 2023. "Spatial Profiling Technologies Illuminate the Tumor Microenvironment." *Cancer Cell* 41 (3): 404–20. https://doi.org/10.1016/j.ccell.2023.01.010.
26. Enguita, Jose María, Ignacio Díaz, Diego García, Tamara Cubiella, María-Dolores Chiara, and Nuria Valdés. 2023. "Visual Analytics Identifies Key MiRNAs for Differentiating Peripancreatic Paraganglioma and Pancreatic Neuroendocrine Tumors." *Frontiers in Endocrinology* 14:1162725. https://doi.org/10.3389/fendo.2023.1162725.
27. Fan, X, Z Mao, D He, C Liao, X Jiang, N Lei, B Hu, et al. 2015. "Expression of Somatostatin Receptor Subtype 2 in Growth Hormone-Secreting Pituitary Adenoma and the Regulation of MiR-185." *Journal of Endocrinological Investigation* 38 (10): 1117–28. https://doi.org/10.1007/s40618-015-0306-7.
28. Glabman, Raisa A, Peter L Choyke, and Noriko Sato. 2022. "Cancer-Associated Fibroblasts: Tumorigenicity and Targeting for Cancer Therapy." *Cancers* 14 (16). https://doi.org/10.3390/cancers14163906.
29. Gossing, Wilhelm, Marcus Frohme, and Lars Radke. 2020. "Biomarkers for Liquid Biopsies of Pituitary Neuroendocrine Tumors." *Biomedicines* 8 (6). https://doi.org/10.3390/biomedicines8060148.
30. Goto, A, T Niki, Y Terado, J Fukushima, and M Fukayama. 2004. "Prevalence of CD99 Protein Expression in Pancreatic Endocrine Tumours (PETs)." *Histopathology* 45 (4): 384–92. https://doi.org/10.1111/j.1365-2559.2004.01967.x.
31. Guinn, Samantha, Brayan Perez, Joseph A Tandurella, Mili Ramani, Jae W

Lee, Daniel J Zabransky, Emma Kartalia, et al. 2025. “Cancer Associated Fibroblasts Drive Epithelial to Mesenchymal Transition and Classical to Basal Change in Pancreatic Ductal Adenocarcinoma Cells with Loss of IL-8 Expression.” *BioRxiv : The Preprint Server for Biology*, January. https://doi.org/10.1101/2025.01.07.631784.

32. Gümürdü, Alican, Ramazan Yildiz, Erden Eren, Gökhan Karakülah, Turgay Ünver, Şermin GENÇ, and Yongsoo Park. 2017. “MicroRNA Exocytosis by Large Dense-Core Vesicle Fusion.” *Scientific Reports* 7 (1): 45661. https://doi.org/10.1038/srep45661.
33. Hackeng, Wenzel M, Lodewijk A A Brosens, Joo Young Kim, Roderick O’Sullivan, You-Na Sung, Ta-Chiang Liu, Dengfeng Cao, et al. 2022. “Non-Functional Pancreatic Neuroendocrine Tumours: ATRX/DAXX and Alternative Lengthening of Telomeres (ALT) Are Prognostically Independent from ARX/PDX1 Expression and Tumour Size.” *Gut* 71 (5): 961–73. https://doi.org/10.1136/gutjnl-2020-322595.
34. He, Zongze, Longyi Chen, Xiao Hu, Jian Tang, Linfu He, Junting Hu, Fan Fei, and Qi Wang. 2019. “Next-Generation Sequencing of MicroRNAs Reveals a Unique Expression Pattern in Different Types of Pituitary Adenomas.” *Endocrine Journal* 66 (8): 709–22. https://doi.org/10.1507/endocrj.EJ18-0487.
35. Hong, Xiafei, Sitan Qiao, Fuqiang Li, Wenze Wang, Rui Jiang, Huanwen Wu, Hao Chen, et al. 2020. “Whole-Genome Sequencing Reveals Distinct Genetic Bases for Insulinomas and Non-Functional Pancreatic Neuroendocrine Tumours: Leading to a New Classification System.” *Gut* 69 (5): 877–87. https://doi.org/10.1136/gutjnl-2018-317233.
36. Iglesias, Pedro. 2023. “Aggressive and Metastatic Pituitary Neuroendocrine Tumors: Therapeutic Management and Off-Label Drug Use.” *Journal of Clinical Medicine* 13 (1). https://doi.org/10.3390/jcm13010116.
37. Kasuki, Leandro, and Gérald Raverot. 2020. “Definition and Diagnosis of Aggressive Pituitary Tumors.” *Reviews in Endocrine & Metabolic Disorders* 21 (2): 203–8. https://doi.org/10.1007/s11154-019-09531-x.
38. Kim, Haedong, Young-Yoon Lee, and V Narry Kim. 2025. “The Biogenesis and Regulation of Animal MicroRNAs.” *Nature Reviews Molecular Cell Biology* 26 (4): 276–96. https://doi.org/10.1038/s41580-024-00805-0.
39. Klöppel, Günter. 2017. “Neuroendocrine Neoplasms: Dichotomy, Origin and Classifications.” *Visceral Medicine* 33 (5): 324–30. https://doi.org/10.1159/000481390.
40. Konukiewitz, Björn, Moritz Jesinghaus, Katja Steiger, Anna Melissa Schlitter, Atsuko Kasajima, Bence Sipos, Giuseppe Zamboni, Wilko Weichert, Nicole Pfarr, and Günter Klöppel. 2018. “Pancreatic Neuroendocrine Carcinomas Reveal a Closer Relationship to Ductal Adenocarcinomas than to Neuroendocrine Tumors G3.” *Human Pathology* 77 (July):70–79. https://doi.org/10.1016/j.humpath.2018.03.018.
41. Kozomara, Ana, Maria Birgaoanu, and Sam Griffiths-Jones. 2019. “MiRBase:

From MicroRNA Sequences to Function." *Nucleic Acids Research* 47 (D1): D155–62. https://doi.org/10.1093/nar/gky1141.
42. Kuzuoğlu-Öztürk, Duygu, Dipankar Bhandari, Eric Huntzinger, Maria Fauser, Sigrun Helms, and Elisa Izaurralde. 2016. "MiRISC and the CCR4-NOT Complex Silence MRNA Targets Independently of 43S Ribosomal Scanning." *The EMBO Journal* 35 (11): 1186–1203. https://doi.org/10.15252/embj.201592901.
43. Lai, Ting-Yu, Tsai-Chen Chiang, Chih-Yuan Lee, Ting-Chun Kuo, Chien-Hui Wu, Yi-Ing Chen, Chun-Mei Hu, et al. 2024. "Unraveling the Impact of Cancer-Associated Fibroblasts on Hypovascular Pancreatic Neuroendocrine Tumors." *British Journal of Cancer* 130 (7): 1096–1108. https://doi.org/10.1038/s41416-023-02565-8.
44. Li, Mingxing, Zhihui Wang, Lixu Zhu, Yifang Shui, Shuijun Zhang, and Wenzhi Guo. 2021. "Down-Regulation of RBP4 Indicates a Poor Prognosis and Correlates with Immune Cell Infiltration in Hepatocellular Carcinoma." *Bioscience Reports* 41 (4). https://doi.org/10.1042/BSR20210328.
45. Love, Michael I, Wolfgang Huber, and Simon Anders. 2014. "Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data with DESeq2." *Genome Biology* 15 (12): 550. https://doi.org/10.1186/s13059-014-0550-8.
46. MANDEL, P, and P METAIS. 1948. "[Nuclear Acids In Human Blood Plasma]." *Comptes rendus des seances de la Societe de biologie et de ses filiales* 142 (3–4): 241–43.
47. Maneshi, Parniyan, James Mason, Mitesh Dongre, and Daniel Öhlund. 2021. "Targeting Tumor-Stromal Interactions in Pancreatic Cancer: Impact of Collagens and Mechanical Traits." *Frontiers in Cell and Developmental Biology* 9:787485. https://doi.org/10.3389/fcell.2021.787485.
48. Megnis, Kaspars, Raitis Peculis, Vita Rovite, Pola Laksa, Helvijs Niedra, Inga Balcere, Olivija Caune, et al. 2019. "Evaluation of the Possibility to Detect Circulating Tumor DNA From Pituitary Adenoma." *Frontiers in Endocrinology* 10:615. https://doi.org/10.3389/fendo.2019.00615.
49. Mhawech-Fauceglia, Paulette, Li Yan, Maryam Sharifian, Xing Ren, Song Liu, Grace Kim, Simon A Gayther, Tanja Pejovic, and Kate Lawrenson. 2015. "Stromal Expression of Fibroblast Activation Protein Alpha (FAP) Predicts Platinum Resistance and Shorter Recurrence in Patients with Epithelial Ovarian Cancer." *Cancer Microenvironment : Official Journal of the International Cancer Microenvironment Society* 8 (1): 23–31. https://doi.org/10.1007/s12307-014-0153-7.
50. Molitch, Mark E. 2017. "Diagnosis and Treatment of Pituitary Adenomas: A Review." *JAMA* 317 (5): 516–24. https://doi.org/10.1001/jama.2016.19699.
51. Muchlińska, Anna, Anna Nagel, Marta Popęda, Jolanta Szade, Magdalena Niemira, Jacek Zieliński, Jarosław Skokowski, Natalia Bednarz-Knoll, and Anna J Żaczek. 2022. "Alpha-Smooth Muscle Actin-Positive Cancer-Associated Fibroblasts Secreting Osteopontin Promote Growth of Luminal Breast Cancer." *Cellular & Molecular Biology Letters*. England.

https://doi.org/10.1186/s11658-022-00351-7.
52. Németh, Kinga, Ottó Darvasi, István Likó, Nikolette Szücs, Sándor Czirják, Lilla Reiniger, Borbála Szabó, et al. 2019. "Comprehensive Analysis of Circulating MiRNAs in the Plasma of Patients With Pituitary Adenomas." *The Journal of Clinical Endocrinology and Metabolism* 104 (9): 4151–68. https://doi.org/10.1210/jc.2018-02479.
53. Oronsky, Bryan, Patrick C. Ma, Daniel Morgensztern, and Corey A. Carter. 2017. "Nothing But NET: A Review of Neuroendocrine Tumors and Carcinomas." *Neoplasia (United States)*. Neoplasia Press, Inc. https://doi.org/10.1016/j.neo.2017.09.002.
54. Peculis, Raitis, Helvijs Niedra, and Vita Rovite. 2021. "Large Scale Molecular Studies of Pituitary Neuroendocrine Tumors: Novel Markers, Mechanisms and Translational Perspectives." *Cancers* 13 (6). https://doi.org/10.3390/cancers13061395.
55. Petracci, Elisabetta, Luigi Pasini, Milena Urbini, Enriqueta Felip, Franco Stella, Fabio Davoli, Maurizio Salvi, et al. 2024. "Circulating Cell-Free and Extracellular Vesicles-Derived MicroRNA as Prognostic Biomarkers in Patients with Early-Stage NSCLC: Results from RESTING Study." *Journal of Experimental & Clinical Cancer Research : CR* 43 (1): 241. https://doi.org/10.1186/s13046-024-03156-y.
56. Ping, Qinrong, Ruping Yan, Xin Cheng, Wenju Wang, Yiming Zhong, Zongliu Hou, Yunqiang Shi, Chunhui Wang, and Ruhong Li. 2021. "Cancer-Associated Fibroblasts: Overview, Progress, Challenges, and Directions." *Cancer Gene Therapy* 28 (9): 984–99. https://doi.org/10.1038/s41417-021-00318-4.
57. Pozniak, Tetiana, Dzmitry Shcharbin, and Maria Bryszewska. 2022. "Circulating MicroRNAs in Medicine." *International Journal of Molecular Sciences* 23 (7). https://doi.org/10.3390/ijms23073996.
58. Radisky, Evette S, and Derek C Radisky. 2010. "Matrix Metalloproteinase-Induced Epithelial-Mesenchymal Transition in Breast Cancer." *Journal of Mammary Gland Biology and Neoplasia* 15 (2): 201–12. https://doi.org/10.1007/s10911-010-9177-x.
59. Rani, Varsha, and Rakesh Singh Sengar. 2022. "Biogenesis and Mechanisms of MicroRNA-Mediated Gene Regulation." *Biotechnology and Bioengineering* 119 (3): 685–92. https://doi.org/10.1002/bit.28029.
60. Reccia, Isabella, Madhava Pai, Jayant Kumar, Duncan Spalding, and Andrea Frilling. 2023. "Tumour Heterogeneity and the Consequent Practical Challenges in the Management of Gastroenteropancreatic Neuroendocrine Neoplasms." *Cancers* 15 (6). https://doi.org/10.3390/cancers15061861.
61. Rindi, Guido, Ozgur Mete, Silvia Uccella, Olca Basturk, Stefano La Rosa, Lodewijk A A Brosens, Shereen Ezzat, et al. 2022. "Overview of the 2022 WHO Classification of Neuroendocrine Neoplasms." *Endocrine Pathology* 33 (1): 115–54. https://doi.org/10.1007/s12022-022-09708-2.
62. Rovite, Vita, Yael Wolff-Sagi, Linda Zaharenko, Liene Nikitina-Zake, Elmars

Grens, and Janis Klovins. 2018. “Genome Database of the Latvian Population (LGDB): Design, Goals, and Primary Results.” *Journal of Epidemiology* 28 (8): 353–60. https://doi.org/10.2188/jea.JE20170079.

63. Saha, Subbroto Kumar, S M Riazul Islam, Tripti Saha, Afsana Nishat, Polash Kumar Biswas, Minchan Gil, Lewis Nkenyereye, Shaker El-Sappagh, Md Saiful Islam, and Ssang-Goo Cho. 2021. “Prognostic Role of EGR1 in Breast Cancer: A Systematic Review.” *BMB Reports* 54 (10): 497–504. https://doi.org/10.5483/BMBRep.2021.54.10.087.
64. Sculthorpe, Declan J, Amy Denton, Wakkas Fadhil, Dewi Rusnita, Mohammad Ilyas, and Abhik Mukherjee. 2025. “High α-SMA Expression in the Tumor Stroma Is Associated with Adverse Clinical Parameters in Mismatch Repair-Proficient Colorectal Cancers Only.” *American Journal of Clinical Pathology* 163 (3): 464–72. https://doi.org/10.1093/ajcp/aqae145.
65. Shah, Manish A, Gyorgy Bodoky, Alexander Starodub, David Cunningham, Desmond Yip, Zev A Wainberg, Johanna Bendell, et al. 2021. “Phase III Study to Evaluate Efficacy and Safety of Andecaliximab With MFOLFOX6 as First-Line Treatment in Patients With Advanced Gastric or GEJ Adenocarcinoma (GAMMA-1).” *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology* 39 (9): 990–1000. https://doi.org/10.1200/JCO.20.02755.
66. Shan, Tao, Shuo Chen, Xi Chen, Wan Run Lin, Wei Li, Jiancang Ma, Tao Wu, et al. 2017. “Cancer-Associated Fibroblasts Enhance Pancreatic Cancer Cell Invasion by Remodeling the Metabolic Conversion Mechanism.” *Oncology Reports* 37 (4): 1971–79. https://doi.org/10.3892/or.2017.5479.
67. Stejskal, Pavel, Hani Goodarzi, Josef Srovnal, Marián Hajdúch, Laura J van ’t Veer, and Mark Jesus M Magbanua. 2023. “Circulating Tumor Nucleic Acids: Biology, Release Mechanisms, and Clinical Relevance.” *Molecular Cancer* 22 (1): 15. https://doi.org/10.1186/s12943-022-01710-w.
68. Sultana, Qamar, Jill Kar, Amogh Verma, Shreya Sanghvi, Nirja Kaka, Neil Patel, Yashendra Sethi, Hitesh Chopra, Mohammad Amjad Kamal, and Nigel H Greig. 2023. “A Comprehensive Review on Neuroendocrine Neoplasms: Presentation, Pathophysiology and Management.” *Journal of Clinical Medicine* 12 (15). https://doi.org/10.3390/jcm12155138.
69. Szklarczyk, Damian, Rebecca Kirsch, Mikaela Koutrouli, Katerina Nastou, Farrokh Mehryary, Radja Hachilif, Annika L Gable, et al. 2023. “The STRING Database in 2023: Protein-Protein Association Networks and Functional Enrichment Analyses for Any Sequenced Genome of Interest.” *Nucleic Acids Research* 51 (D1): D638–46. https://doi.org/10.1093/nar/gkac1000.
70. Takahashi, Daigoro, Motohiro Kojima, Toshihiro Suzuki, Motokazu Sugimoto, Shin Kobayashi, Shinichiro Takahashi, Masaru Konishi, et al. 2018. “Profiling the Tumour Immune Microenvironment in Pancreatic Neuroendocrine Neoplasms with Multispectral Imaging Indicates Distinct Subpopulation Characteristics Concordant with WHO 2017 Classification.” *Scientific Reports* 8 (1): 1–10. https://doi.org/10.1038/s41598-018-31383-9.

71. Thul, Peter J, and Cecilia Lindskog. 2018. “The Human Protein Atlas: A Spatial Map of the Human Proteome.” *Protein Science : A Publication of the Protein Society* 27 (1): 233–44. https://doi.org/10.1002/pro.3307.
72. Toma, Iustin Daniel, Dan Alexandru Niculescu, Simona Andreea Găloiu, Raluca Alexandra Trifănescu, and Cătălina Poiană. 2025. “Comparative Mortality in Pituitary Adenomas Subtypes: A Tertiary Referral Center Study.” *Endocrine* 87 (2): 782–87. https://doi.org/10.1007/s12020-024-04073-y.
73. Valassi, Elena, Natalia García-Giralt, Jorge Malouf, Iris Crespo, Jaume Llauger, Adolfo Díez-Pérez, and Susan M Webb. 2019. “Circulating MiR-103a-3p and MiR-660-5p Are Associated with Bone Parameters in Patients with Controlled Acromegaly.” *Endocrine Connections* 8 (1): 39–49. https://doi.org/10.1530/EC-18-0482.
74. Vathiotis, Ioannis A, Myrto K Moutafi, Prajan Divakar, Thazin Nwe Aung, Tao Qing, Aileen Fernandez, Vesal Yaghoobi, et al. 2021. “Alpha-Smooth Muscle Actin Expression in the Stroma Predicts Resistance to Trastuzumab in Patients with Early-Stage HER2-Positive Breast Cancer.” *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research* 27 (22): 6156–63. https://doi.org/10.1158/1078-0432.CCR-21-2103.
75. Vetrivel, Sharmilee, Ru Zhang, Mareen Engel, Barbara Altieri, Leah Braun, Andrea Osswald, Martin Bidlingmaier, et al. 2021. “Circulating MicroRNA Expression in Cushing’s Syndrome.” *Frontiers in Endocrinology* 12:620012. https://doi.org/10.3389/fendo.2021.620012.
76. Virolle, T, E D Adamson, V Baron, D Birle, D Mercola, T Mustelin, and I de Belle. 2001. “The Egr-1 Transcription Factor Directly Activates PTEN during Irradiation-Induced Signalling.” *Nature Cell Biology* 3 (12): 1124–28. https://doi.org/10.1038/ncb1201-1124.
77. Visser, Karin E de, and Johanna A Joyce. 2023. “The Evolving Tumor Microenvironment: From Cancer Initiation to Metastatic Outgrowth.” *Cancer Cell* 41 (3): 374–403. https://doi.org/10.1016/j.ccell.2023.02.016.
78. Wang, Baiyao, Wei Liu, Chunshan Liu, Kunpeng Du, Zhaoze Guo, Guoqian Zhang, Zhong Huang, et al. 2023. “Cancer-Associated Fibroblasts Promote Radioresistance of Breast Cancer Cells via the HGF/c-Met Signaling Pathway.” *International Journal of Radiation Oncology, Biology, Physics* 116 (3): 640–54. https://doi.org/10.1016/j.ijrobp.2022.12.029.
79. Wang, Bin, Hanfei Guo, Hongquan Yu, Yong Chen, Haiyang Xu, and Gang Zhao. 2021. “The Role of the Transcription Factor EGR1 in Cancer.” *Frontiers in Oncology* 11:642547. https://doi.org/10.3389/fonc.2021.642547.
80. Wang, Chong, and Hui Liu. 2022. “Factors Influencing Degradation Kinetics of MRNAs and Half-Lives of MicroRNAs, CircRNAs, LncRNAs in Blood in Vitro Using Quantitative PCR.” *Scientific Reports* 12 (1): 7259. https://doi.org/10.1038/s41598-022-11339-w.
81. Waters, Andrew M, and Channing J Der. 2018. “KRAS: The Critical Driver and Therapeutic Target for Pancreatic Cancer.” *Cold Spring Harbor*

Perspectives in Medicine 8 (9). https://doi.org/10.1101/cshperspect.a031435.
82. White, Bruce A, and Susan P B T - Endocrine and Reproductive Physiology (Fourth Edition) Porterfield, eds. 2013. "5 - Hypothalamus-Pituitary Complex." In , 99-e2. Philadelphia: Mosby. https://doi.org/https://doi.org/10.1016/B978-0-323-08704-9.00005-1.
83. Wiechec, Emilia, Mustafa Magan, Natasa Matic, Anna Ansell-Schultz, Matti Kankainen, Outi Monni, Ann-Charlotte Johansson, and Karin Roberg. 2021. "Cancer-Associated Fibroblasts Modulate Transcriptional Signatures Involved in Proliferation, Differentiation and Metastasis in Head and Neck Squamous Cell Carcinoma." *Cancers* 13 (13). https://doi.org/10.3390/cancers13133361.
84. Wu, Lijun, Ke Li, Wei Lin, Jianjiang Liu, Qiang Qi, Guoliang Shen, Weixin Chen, and Wenjun He. 2022. "Long Noncoding RNA LINC01291 Promotes the Aggressive Properties of Melanoma by Functioning as a Competing Endogenous RNA for MicroRNA-625-5p and Subsequently Increasing IGF-1R Expression." *Cancer Gene Therapy* 29 (3–4): 341–57. https://doi.org/10.1038/s41417-021-00313-9.
85. Wu, Zerui, Lin Cai, Jianglong Lu, Cheng De Wang, Jiaqing Guan, Xianbin Chen, Jinsen Wu, et al. 2018. "MicroRNA-93 Mediates Cabergoline-Resistance by Targeting ATG7 in Prolactinoma." *The Journal of Endocrinology*, September. https://doi.org/10.1530/JOE-18-0203.
86. Xu, Maosen, Tao Zhang, Ruolan Xia, Yuquan Wei, and Xiawei Wei. 2022. "Targeting the Tumor Stroma for Cancer Therapy." *Molecular Cancer* 21 (1): 208. https://doi.org/10.1186/s12943-022-01670-1.
87. Ye, Zeng, Qiang Li, Yuheng Hu, Haifeng Hu, Junfeng Xu, Muzi Guo, Wuhu Zhang, et al. 2024. "The Stromal Microenvironment Endows Pancreatic Neuroendocrine Tumors with Spatially Specific Invasive and Metastatic Phenotypes." *Cancer Letters* 588 (April):216769. https://doi.org/10.1016/j.canlet.2024.216769.
88. Yin, Weiling, and Andrea C Gore. 2010. "The Hypothalamic Median Eminence and Its Role in Reproductive Aging." *Annals of the New York Academy of Sciences* 1204 (August):113–22. https://doi.org/10.1111/j.1749-6632.2010.05518.x.
89. Yoon, Hyunho, Chih Min Tang, Sudeep Banerjee, Antonio L. Delgado, Mayra Yebra, Jacob Davis, and Jason K. Sicklick. 2021. "TGF-B1-Mediated Transition of Resident Fibroblasts to Cancer-Associated Fibroblasts Promotes Cancer Metastasis in Gastrointestinal Stromal Tumor." *Oncogenesis* 10 (2). https://doi.org/10.1038/s41389-021-00302-5.
90. Zacharjasz, Julian, Marta Sztachera, Michał Smuszkiewicz, and Monika Piwecka. 2024. "Micromanaging the Neuroendocrine System - A Review on MiR-7 and the Other Physiologically Relevant MiRNAs in the Hypothalamic-Pituitary Axis." *FEBS Letters* 598 (13): 1557–75. https://doi.org/10.1002/1873-3468.14948.
91. Zampetti, Benedetta, Erika Grossrubatscher, Paolo Dalino Ciaramella, Edoardo Boccardi, and Paola Loli. 2016. "Bilateral Inferior Petrosal Sinus

Sampling." *Endocrine Connections* 5 (4): R12-25. https://doi.org/10.1530/EC-16-0029.
92. Zhang, Hao, Xinghai Yue, Zhe Chen, Chao Liu, Wantao Wu, Nan Zhang, Zaoqu Liu, et al. 2023. "Define Cancer-Associated Fibroblasts (CAFs) in the Tumor Microenvironment: New Opportunities in Cancer Immunotherapy and Advances in Clinical Trials." *Molecular Cancer* 22 (1): 159. https://doi.org/10.1186/s12943-023-01860-5.
93. Zhao, Zehua, Tianming Li, Yuan Yuan, and Yanmei Zhu. 2023. "What Is New in Cancer-Associated Fibroblast Biomarkers?" *Cell Communication and Signaling : CCS* 21 (1): 96. https://doi.org/10.1186/s12964-023-01125-0.