REGULATORY NETWORK-DRIVEN BIOMARKER DISCOVERY FOR PANCREATIC DUCTAL CARCINOMA AND PANCREATIC PSEUDOCYST

THESIS

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IN
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CANDIDATE'S DECLARATION

I, Sejal (24/MSCBIO/37), hereby certify that the work which is being presented in the thesis entitled "Regulatory Network-Driven Biomarker Discovery for Pancreatic Ductal Carcinoma and Pancreatic Pseudocyst" in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University, is an authentic record of my own work carried out during the period from 2024 to 2026 under the supervision of Dr. Asmita Das, Associate Professor, Department of Biotechnology. The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

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Abstract

Pancreatic ductal carcinoma (PDAC) is one of the most aggressive cancers that impact the pancreas. human pancreas. It has a dismal prognosis which is largely due to the fact that there are no early warning signs of the disease, An insidious course and often confusing diagnosis because of overlap in clinical features with non-malignant conditions. Although benign, pancreatic pseudocyst is a condition that is especially. also challenging in this aspect as it may have imaging and clinical characteristics that are similar to other conditions. Are similar to PDAC, creating diagnostic confusion. This thesis is a continuation of this final. A research paper was converted to a thorough research into systems biology focusing on regulatory Network analysis is proposed as a method to identify biomarkers in the two pancreatic diseases.

The genes related to PDAC and pseudocyst of pancreas were obtained individually from the The comparative toxicogenomics database (CTD) was used to determine the degree of overlap and it was evaluated using the Comparative Toxicogenomics Database. intersection analysis with the other one has resulted in the identification of 31 common genes. These are regular genes, which were the basis for We are building the miRNA-gene and the transcription factor (TF) to gene connections, and also creating data on the pancreas tissue-specific connections between them. The analysis was carried out in regulatory networks with the NetworkAnalyst platform. These were topologically examined by examining them as follows: Based on the networks constructed using degree centrality, we identified hsa-miR-34a-5p, hsa-miR-16-5p and hsa-miR-191-5p. The top miRNA hubs are 335-5p, and MYC, CCND1, VEGFA, RELA and CDKN1B. TFs and regulatory genes that stood out as prominent TFs or gene regulation hubs. In functional analysis, central nodes like the ones presented here are the ones around which the other nodes revolve. The roles they play in cell cycle control, resistance to apoptosis, inflammatory and antimicrobial response, regulation of metabolism, and matrix remodeling were all highlighted. signaling, the neovascularization process and dynamics of the tumor microenvironment.

The results indicate that there may be a possibility of pairing some tumor-

suppressive miRNA hubs with oncogenic TF hubs. Increased levels of these could also predict the nature of the growth, and be included in a multi-marker diagnostic panel that can distinguish malignant from nonmalignant. Benign pancreatic pseudocyst: PDAC. Because this present work is a completely computational work, experimental validation with patient-derived patient tissue, plasma or serum miRNA samples, An independent clinical datasets is still needed. However, the regulatory network. a biologically-inspired and interpretable starting point for the development of such a framework is provided here. Future work in development of pancreatic disease biomarkers.

Keywords: Pancreatic ductal carcinoma, pancreatic pseudocyst, NetworkAnalyst, Areas of interest include microRNA, transcription factors, diagnostic biomarkers, systems biology, and regulatory network.

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
PDAC	Pancreatic ductal carcinoma
CTD	Comparative Toxicogenomics Database
miRNA	microRNA
TF	Transcription factor
MYC	MYC proto-oncogene
CCND1	Cyclin D1
VEGFA	Vascular endothelial growth factor A
RELA	RELA proto-oncogene, NF-kappa-B subunit
CDKN1B	Cyclin-dependent kinase inhibitor 1B
BCL2L1	BCL2-like 1
FAS	Fas cell surface death receptor
POU5F1	POU class 5 homeobox 1
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
EMT	Epithelial-mesenchymal transition
ROC	Receiver operating characteristic
qRT-PCR	Quantitative reverse-transcription polymerase chain reaction

Chapter 1

INTRODUCTION

The Chapter provides the background in terms of biology and diagnosis of the study. It introduces The need for network-based pancreatic ductal carcinoma, pancreatic pseudocyst and considered biomarker discovery.

1.1 Background of Pancreatic Disease

The pancreas is a complex organ, with both endocrine and exocrine functions. An organ with a wide clinical range of disease symptoms. Many pancreatic Signs and symptoms, imaging appearance and histopathology are alike in both conditions. Differential diagnosis is difficult because they have inflammatory markers. Among such In particular, there are two worth of special mention, PDAC and pancreatic pseudocyst. The latter is a benign fluid while the former is an aggressive malignancy, if caught when it is advanced. Collection that has developed as a result of pancreatitis. In spite of their very different character, Abdominal pain, structural changes in the pancreas and similar radiological changes can occur in both. signals. Chromatin remodeling is a key factor in the retrieval of memory in the brain.The retrieval of memory in the brain depends on common and different molecular regulators involved in chromatin remodeling. In both of these situations, these two conditions can have a relevant impact on diagnostic accuracy.

Often, pancreatic diseases do not occur as a result of a single molecular fault. Rather, they reflect disrupted a cell cycle pathway at several levels, such as the cell cycle machinery, pro-survival signals and angiogenic pathways as well as inflammatory cascades. Representing these As a networked ensemble of

interconnected molecules, it becomes possible to make a more structured and A meaningful, biological strategy to identifying candidate biomarkers.

1.2 Pancreatic Ductal Carcinoma

PDAC is responsible for the overwhelming majority of pancreatic cancer cases and continues to carry one of the poorest survival outcomes across all solid malignancies. As highlighted in the research paper, the clinical silence of PDAC in its early stages directly correlates with late presentation and reduced treatment options. At the molecular level, the disease is driven by unchecked cellular proliferation, evasion of programmed cell death, activation of inflammatory pathways, development of new vasculature, complex stromal interactions, and metabolic reprogramming. Given how difficult early detection remains, biomarkers that authentically capture these biological features are critical for telling apart malignant disease from inflammatory but benign pancreatic conditions.

Single gene/molecule approaches are often not powerful enough to capture The only difficulty faced in the study of pancreatic disease biology. A regulatory network perspective, Contrastingly, views genes, miRNAs and transcription factors as part of a larger, interconnected system. This type of presentation is very well suited to disease states in which the clinical manifestation is most evident. Many molecular axes are dysregulated concurrently to give rise to the phenotype. Identifying hub regulators, the ones that affect a lot of targets, would be better More diagnostic utility than any any molecule taken alone.

This thesis will attempt to expand on the final research paper and make it a completed work. Academic research showing the link between shared regulatory biomarkers in PDAC and that in oral cancer. There are systems biology methods that can be used to identify pancreatic pseudocyst. The work specifically focuses on the common gene set obtained from CTD, building of miRNA gene and TF-gene regulatory networks, the integration of pancreas tissue-specific interaction Data and biological interpretation of the obtained regulatory hubs. The present work is of a systems level approach as pancreatic pathologies are rare, Caused by only one molecular change. Rather, they comprise layers of changes in cell-cycle. Genes, inflammatory mediators, angiogenic regulators and apoptosis-associated molecules.

1.3 Pancreatic Pseudocyst and Diagnostic Ambiguity

Pancreatic pseudocyst is a walled fluid collection, which usually develops after a pancreatitis attack. pancreatitis. Though it doesn't cause cancer, its clinical and radiological presentation can be similar to cancer and this is why it is important to understand. The result is a picture that is very similar to a problem that the research paper places at its heart: PDAC.The picture looks very like that of PDAC, whom the research paper places at the heart of its problems. diagnostic inquiry. If there is an inflammatory lesion that causes fluid to accumulate in the nearby tissues, Sometimes standard imaging is unable to distinguish a pseudocyst from a tumor in a remodeling, especially when the tumor is located near the wall and/or adjacent to other fluids. malignant lesion. In such cases, molecular biomarkers that can complement clinical diagnosis can prove useful. Examinations of the and imaging results may be important in facilitating proper diagnosis.

The present work is done in a systems level approach because pathologies that of the pancreas are hardly envisaged in isolation. Can be explained with only one molecule change. On the contrary, these involve cascading changes in cell-cycle. Genes, inflammatory mediators, angiogenic regulators and apoptosis associated molecules. By organizing these molecules into interpretable networks, the study provides a structured route for biomarker prioritization.

1.4 Need for Network-Based Biomarker Discovery

Approaches that focus on a single gene or molecule at a time are often ill-equipped to capture the complexity inherent in pancreatic disease biology. A regulatory network perspective, by contrast, treats genes, miRNAs, and transcription factors as components of a larger, interconnected system. This framing is particularly appropriate for diseases where the clinical phenotype emerges from simultaneous dysregulation across many molecular axes. Identifying hub regulators — those that influence a large number of targets — offers greater diagnostic leverage than any individual molecule considered in isolation.

The present work follows a systems-level approach because pancreatic pathologies are rarely explained by a single molecular alteration. Instead, they involve layered changes in cell-cycle genes, inflammatory mediators, angiogenic regu-

lators and apoptosis-associated molecules. By organizing these molecules into interpretable networks, the study provides a structured route for biomarker prioritization.

1.5 Aim of the Thesis

This thesis aims to build upon the final research paper by developing it into a fully elaborated academic study that demonstrates how shared regulatory biomarkers between PDAC and pancreatic pseudocyst can be identified through systems biology methods. The work specifically centers on the common gene set derived from CTD, the construction of miRNA-gene and TF-gene regulatory networks, the integration of pancreas tissue-specific interaction data, and the biological interpretation of the resulting regulatory hubs.

The present work follows a systems-level approach because pancreatic pathologies are rarely explained by a single molecular alteration. Instead, they involve layered changes in cell-cycle genes, inflammatory mediators, angiogenic regulators and apoptosis-associated molecules. By organizing these molecules into interpretable networks, the study provides a structured route for biomarker prioritization.

1.6 Objectives

The study pursues several defined objectives: retrieval of disease-associated gene sets for both PDAC and pancreatic pseudocyst from CTD; identification of genes shared between the two conditions; construction of miRNA-gene and TF-gene regulatory interaction networks; prioritization of hub miRNAs and transcription factors based on degree centrality; biological interpretation of these hubs in the context of pancreatic disease; and the proposal of a candidate diagnostic biomarker panel for subsequent experimental and clinical validation.

The present work follows a systems-level approach because pancreatic pathologies are rarely explained by a single molecular alteration. Instead, they involve layered changes in cell-cycle genes, inflammatory mediators, angiogenic regulators and apoptosis-associated molecules.

Chapter 2

LITERATURE REVIEW

This chapter reviews the conceptual foundation required to interpret the work. It describes pancreatic cancer biology, pseudocyst-related ambiguity, miRNA regulation, transcription factor control and systems-biology approaches.

2.1 Biological Features of PDAC

The ductal epithelium of the pancreas gives rise to PDAC which passes through a series of stages. Of the complex molecular process. It has an extremely aggressive nature, which is associated with a fast invasion of Circumstances where the surrounding tissue is damaged, there is a dense stromal reaction, escape immune surveillance, Systemic therapy is limited in penetration and there is a long asymptomatic period. Molecularly, The disease is characterized by disregulated proliferation, apoptosis, inflammatory and immune responses. Themes directly echoed in the hub regulators include signaling, as well as angiogenesis. The research paper brings certain genes to light, among them MYC, the gene of interest for cancer, as well as CCND1, VEGFA, and RELA.

This biological context is the foundation for the aim of the present thesis: although the various groups of individuals studied were: PDAC and pseudocyst are fundamentally different diseases, which may converge. common molecular signals. A network approach takes those shared signals to capture a networked approach. Discover patterns in regulation that are more indicative of diagnosis than any one molecule in isolation.

2.2 Pancreatic Pseudocyst as a Benign Inflammatory Condition

A pancreatic pseudocyst is a benign inflammatory condition accounted for in 20% of cases. Pseudocyst most often occurs after pancreatitis and is considered a type of (gastroenterologic) pseudocyst. Inflammatory injury response at the local level in the pancreatic bed. Although it doesn't have any It is histologically similar to PDAC and has clinical and radiological features that would cause the patient to be at risk of developing a true diagnosis of PDAC. difficulty, particularly if a pancreatic lesion occurs in conjunction with a diffuse, or vague, or not-very-specific, sensation of pain. symptoms. A biomarker, which is able to identify the molecular fingerprint of inflammatory processes, would be excellent for this purpose. The chances of tissue remodeling compared to malignant transformation could make a critical difference in helping to improve the chances for the following. Clinician confidence for clinical decision making.

The literature background justifies the current thesis, which is based on the assumption that PDAC and pancreatic pseudocysts are different in terms of biology, but could share molecular signals. A network-driven approach could take advantage of those common signals to uncover patterns of regulation that are more informative. More so than single molecules, taken individually.

2.3 Role of Common Disease-Associated Genes

A total of 31 genes were detected that were shared between PDAC and pancreatic pseudocyst. research paper. These common genes, its presence, indicates convergent engagement of certain biological processes within the pancreatic microenvironment by both conditions. Genes that are involved in cell-cycle regulation include CCND1, CCND2, CDK6, and others. FAS and BCL2L1 are found on the apoptotic axis. VEGFA introduces the angiogenic aspect, and RELA link inflammation to the survival signals that are downstream.

This literature background is in support of the rationale for the present thesis concerning PDAC and pancreatic cancer. pseudocysts are distinct but can have common molecular signals. A network-driven strategy can leverage those common signals to detect regulatory patterns that are more informative than single molecules taken apart.

2.4 microRNAs in Disease Regulation

MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of genes after transcription is generally carried out by binding to complementary regions of target mRNAs and either Inhibiting their translation and/or inducing their degradation. When it comes to cancer, single miRNAs can have tumour suppressor or oncogenic properties, Depending on their repertoire of interest or target repertoire. Of all the common genes for disease analyzed in this research, hsa-miR-34a-5p, hsa-miR-16-5p and miR-335-5p were the top three most interconnected microRNAs. microRNAs (miRNAs) as a group are implicated in their far-reaching regulation over the molecular mechanisms and signaling pathways of a wide array of biological processes, including development, proliferation, and differentiation. One of the pancreatic pathology pictures.

This literature background is useful to the rationale of this current thesis about PDAC and pancreatic. pseudocyst have unique biological characteristics and sometimes may have shared molecular markers. A network-driven strategy can leverage those common signals to look for more information-rich regulatory patterns more than single molecules may be taken individually..

2.5 hsa-miR-34a-5p

The tumor-suppressive microRNA hsa-miR-34a-5p is widely believed to be such. TF expression patterns and functional targets in cancer. In the network built in the research paper it was the most connected node within the miRNA network with degree 11. Its However, it is hypothesized that regulatory activity will reduce the production of important proliferators including: CCND1, CDK6 and MYC. These targets may become when this suppression is reduced. dysregulated, this makes it conducive to the development of malignant transformation.

The literature background will be used to write the rationale for the current thesis: PDAC and pancreatic pseudocyst are indeed different, but may provide the same molecular signals. A network-driven strategy can leverage those common signals to inform identification of regulatory patterns that are more informative More powerful than individual molecules when they are used alone.

2.6 hsa-miR-16-5p

hsa-miR-16-5p was identified as a second major miRNA hub (with degree 9). Its documented Stimulatory targets include cyclin family members, like CCND1 and CCND2. This miRNA to be a cell cycle regulator of the G1/S checkpoint. Disrupted miR-16-5p This activity might then impact on the inflammatory remodeling typical of pseudocysts, as well as.This activity may then have an impact not just on the inflammatory remodeling typical of pseudocysts, but also. In PDAC progression, uncontrolled proliferation observed in the tumor cells was just as described during formation.

This literature background is in support of the rationale for the current thesis: PDAC and pancreatic While they are biologically distinct, pseudocyst may have similar molecular signals. A network-driven With those common signals strategy can determine regulatory patterns that are more informative. more effective than the effect of individual molecules alone.

2.7 hsa-miR-335-5p

As with miR-16-5p, hsa-miR-335-5p had the same degree value of 9, and was in the same position, A key role in the miRNA-gene network. The research paper makes a connection between this miRNA and genes. Some of the genes involved in these processes, such as POU5F1 and BCL2L1, which are related to stemness and apoptosis respectively. Its regulatory scope may thus encompass phenotypic plasticity as well as the more aggressive behavioral Characteristics that are linked to the onset of pancreatic cancer.

This background in the literature assists to develop the rationale in this thesis: PDAC and pancreatic Biologically, they are different and could share molecular signals; they are called pseudocysts. A network-driven Using those common signals, strategy can reduce the number of bad combinations by identifying patterns in the regulation that are more informative. than in the case of single molecules when analysed separately.

2.8 Transcription Factor Hubs

Transcription factors act as regulators of gene expression, regulating the expression of a whole host of genes. Activity of large groups of downstream targets. In the TF-gene regulatory network shown in MYC, CCND1, VEGFA, RELA and CDKN1B were identified as -regulatory proteins.The proteins, MYC, CCND1, VEGFA, RELA and CDKN1B were identified as -regulatory proteins. high-connectivity regulatory nodes. Some of these don't easily fall into the category of classical, Each of them has a lot of influence on the transcription regulation of a program of genes, transcription factor definition. Applicable to the disease of the pancreas. The density of their high degree values is indicated by their high degree values. Molecular interactions and the possible biological significance.

This literature background is relevant for the current thesis which focuses on PDAC and pancreatic pseudocysts are biologically distinct, but could have the same molecular signals. A network-driven Those common signals can help strategy see patterns in the regulatory system which are more meaningful. more than single molecules – taken alone.

2.9 Systems Biology and NetworkAnalyst

Systems biology is a promising methodology to unravel complex biological processes because of its capability to: Considering interactions of molecular components in larger, organized networks. NetworkAnalyst is a web-based application to build up regulatory networks. It allows to automatically incorporate curated interaction databases and retrieve from user provided gene lists. In this study, NetworkAnalyst serves as the central analytical tool for building the miRNA-gene, TF-gene, and pancreas tissue-specific interaction networks.

This literature background supports the rationale for the current thesis: PDAC and pancreatic pseudocyst are biologically different but may share molecular signals. A network-driven strategy can use those shared signals to identify regulatory patterns that are more informative than single molecules considered independently.

2.10 Hub-Based Biomarker Discovery

Hub nodes in network biology are nodes with a substantially higher number of connections than the rest of the nodes in the network. Sustain in conjunction with other components. They are important because changes in the activity of the hubs that affect. can spread along several different avenues at once and are therefore appealing choices for dissemination via several routes. biomarker studies. It is a good approach to look first for high-degree regulators in the discovery of biomarkers that regulate or are being influenced by an additional high-degree regulator. strategy due to the fact that often they are at the crucial hub of disease biology. However, the status of a computational network hub does not necessarily imply that something is clinically useful. More stringent experimental and clinical testing are still necessary.

This literature background forms the basis for the purpose of the current thesis, which is PDAC and pancreatic While pseudocysts are different types of cysts, they can have similar molecular signals. A network-driven strategy can use those shared signals to identify regulatory patterns that are more informative than single molecules considered independently.

Chapter 3

SAMPLE DATA COLLECTION FORMAT

This is a typical sample of how future validation studies will be conducted. It is included to Enable repeatability and program. Patient-related data should only be collected after: Ethical approval and informed consent.

Field	**Description**
Sample ID	Unique anonymized identifier
Clinical group	PDAC / pancreatic pseudocyst / control
Age and sex	Basic demographic variables
Clinical diagnosis method	Histopathology, imaging, biochemical markers or clinician-confirmed diagnosis
Sample type	Tissue, cyst fluid, serum or plasma
RNA quality metric	Purity and integrity value
miRNA expression	Ct value or normalized expression
Gene expression	Ct value or normalized expression
Final classification	Confirmed diagnostic category

Table 3.1: Sample Data Collection

3.1 Data security

Before analysis all data should be anonymized. The patient's identifiers should not be stored in the analysis file. Raw clinical information must only be accessed by authorized researchers. only.

3.2 Data quality

Any missing values, outliers and batch effects should be noted. Technical replicates can improve reliability. Normalization controls should be carefully chosen for both miRNA and other genes. mRNA assays.

3.3 Reporting

Confidence intervals and validation metrics should be included in the results. Negative findings should also be reported as this could further assist in the development of biomarker panels and further avoid unnecessary reports. overinterpretation.

Chapter 4

MATERIALS AND METHODS

This chapter explains the methodology followed in the study, including data retrieval, common gene identification, regulatory network construction, tissue-specific network integration and hub analysis.

4.1 Study Design

This investigation was conceived as a computational systems-biology study. Beginning with the retrieval of disease-associated genetic data and culminating in the biological interpretation of network hubs, the analysis followed a stepwise workflow: gene set retrieval from a curated database, identification of genes shared between both conditions, construction of regulatory interaction networks, selection of hub candidates using topological measures, and functional interpretation of the selected hubs.

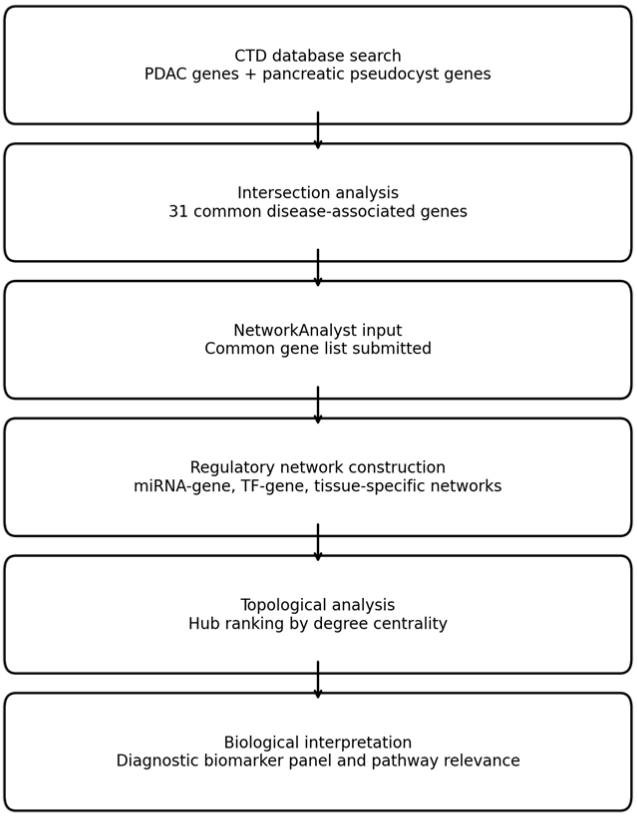

Figure 4.1: **Methodological workflow of the regulatory network-driven biomarker discovery study**

4.2 Data Retrieval from CTD

Genes associated with PDAC and pancreatic pseudocyst were individually retrieved from the Comparative Toxicogenomics Database, chosen for its well-curated collection of disease-gene and chemical-gene interaction data. Distinct gene sets for each condition were downloaded and organized in preparation for comparative analysis.

Each methodological step was carried out with the shared goal of identifying biomarkers that are both topologically central within the regulatory network and interpretable in biological terms. The emphasis in all of this was on being reproducible and on good systematic exploitation of curated data, Clarity of purpose of selection of hubs.

4.3 Common Gene Identification

The two disease-specific gene sets were directly compared and common gene sets were identified. We calculated it to identify the genes linked to PDAC and pancreatic pseudocyst. As Of this, 31 genes were found to overlap and were considered in the following. As shared molecular candidates and forwarded to the regulatory network construction phase.

This step is part of a larger goal of focusing on the identification of biomarkers that are those of the most importance. Computationally significant and biologically meaningful. The method emphasizes Structured data use and transparent hub selection, reproducibility.

4.4 miRNA-Gene Network Construction

From this list of 31 common genes, the list was entered in NetworkAnalyst for making the miRNA gene regulatory network. In this network, nodes could either be genes or miRNAs. and the borders between them indicated regulatory effects that were curated and the rest that were non-curated. predicted data. UBP between them were then calculated for degrees and ranked. To create a network and understand who or what has the highest “hub” potential.

This step assists in the overall goal of determining a set of biomarkers that are priority. Computationally accessible, and biologically meaningful. The method emphasizes reproducible and structured data - and transparent hub selection.

4.5 Transcription Factor-Gene Network Construction

A new TF-gene regulatory network was built in NetworkAnalyst and then imported to the same server to receive further processing. Describe the transcriptomic regulon (TR) of the common gene set. The objective To identify transcription factors and high-degree gene hubs that were located within the disease network. The most common method used for ranking node connectivity was degree centrality.

This step will help to achieve the goal of prioritizing biomarkers that are both Both computationally and biologically interpretable. The method emphasizes Structured data use and clear hub selection is given priority along with reproducibility.

4.6 Pancreas Tissue-Specific Network

To enhance the biological relevance of the results, another layer of pancreas tissue was added to these. Specific interaction data was taken into the analysis. Tissue-specific networks provide A significant contextual filter, which can help researchers with determining if the identified regulators play a role in the context at which you are using them. keep meaningful functions in the microenvironment of the pancreas in particular, but Simply the fact of being widely distributed throughout a few tissues.

This step aims at achieving the target of giving a priority to a set of biomarkers that are Central and meaningful to biological interpretation, computational. The method emphasizes The concepts of reproducibility and structured data use and transparent hub selection.

4.7 Hub Selection Criteria

The method of hub identification was based on the degree centrality as the first criterion — a degree that calculates the number of connecting interaction edges of a node. Following the Based on the research paper, the top three miRNA hubs and the most relevant miRNAs were selected to undertake the approach used in the research paper. The gene hubs that are connected with TF and regulatory hubs

were chosen for further biological investigation. The The most important miRNA hubs identified were hsa-miR-34a-5p, hsa-miR-16-5p and hsa-miR-335-5p. The main TF/regulatory hubs were MYC, CCND1, VEGFA, RELA and CDKN1B.

This step is part of the goal of prioritizing biomarker(s) that are both Readily computable and easily understood. The method emphasizes reproducible data, structured use of data and transparency for the selection of hubs.

4.8 Functional Interpretation

The identified hub molecules were examined for their functional significance and information was obtained from literature sources. made important contributions to cancer and inflammatory biology, specifically in regard to Cell proliferation, cell death, inflammatory signalling and angiogenesis. The orientation of the miRNA The mediated regulatory effects were interpreted according to target relationships that are supported by literature. These are presented in the research paper.

This step is part of the ultimate goal of prioritizing biomarkers that are ”both” Computationally important and biologically relevant. The method emphasizes Data is reproducible and the structures used are transparent and selected based on the hubs.

4.9 Thesis Expansion Method

The final research paper was the main analytical source of this thesis. Chapter Content of structure, organization of front matter, forms of tables and standards of academic writing were looked at. Made all formatting choices — Contents, Introduction, Declaration, Supervisor certificate, title page layout, chapter etc. Please use the following style: headings, 12-point Times New Roman font, and 1.5 line spacing — as per the DTU thesis. template.

This step helps to achieve the overall goal of prioritizing biomarkers that are easily accessible in the computer and accessible to the biology. The method emphasizes structured data use and transparent hub selection, reproducibility.

Tool/Resource	**Purpose**	**Output Used in Thesis**
CTD	Retrieval of disease-associated genes for PDAC and pancreatic pseudocyst	Common disease gene list
NetworkAnalyst	Construction of miRNA-gene, TF-gene and tissue-specific networks	Network maps, hub degree values
Degree centrality	Topological ranking of nodes	Hub miRNAs and TF/regulatory hubs
Literature interpretation	Biological explanation of hubs	Diagnostic relevance and validation needs

Table 4.1: Tools and Data Summary

Chapter 5

RESULTS

This chapter presents the results obtained from the regulatory network-driven analysis. It interprets common genes, miRNA hubs, transcription factor hubs and pancreas tissue-specific network findings.

5.1 Identification of Common Genes

Comparative analysis of the two CTD-derived gene sets revealed 31 genes shared between PDAC and pancreatic pseudocyst. The existence of this common gene pool demonstrates that despite the fundamental difference in disease nature, both conditions recruit overlapping molecular pathways, particularly those governing inflammation, cell-cycle activation, programmed cell death, and blood vessel formation.

Gene	Biological Group	Relevance
CCND1	Cell-cycle progression	Supports proliferation and G1/S transition
CCND2	Cell-cycle progression	Cyclin-mediated control of cell division
CDK6	Cell-cycle kinase	Regulates G1 phase progression
FAS	Apoptosis	Death receptor signaling
BCL2L1	Apoptosis resistance	Cell survival and anti-apoptotic control
CCL2	Inflammation	Chemokine-mediated inflammatory response
MYC	Transcriptional regulation/proliferation	Central regulator of growth and metabolism
VEGFA	Angiogenesis	Tumor vascularization and hypoxia adaptation
RELA	Inflammatory survival signaling	NF-kB pathway component
CDKN1B	Cell-cycle control	Regulator of cell-cycle progression

Table 5.1: Common Genes

5.2 miRNA-Gene Regulatory Network

Construction of the miRNA-gene regulatory network brought three microRNAs to the fore as dominant hubs: hsa-miR-34a-5p with a degree of 11, and hsa-miR-16-5p and hsa-miR-335-5p each with a degree of 9. All three have mainly tumor suppressive properties and are thought to inhibit the expression of genes that inhibit proliferation and prevent the death of cells, sustain inflammation, and promote angiogenesis.

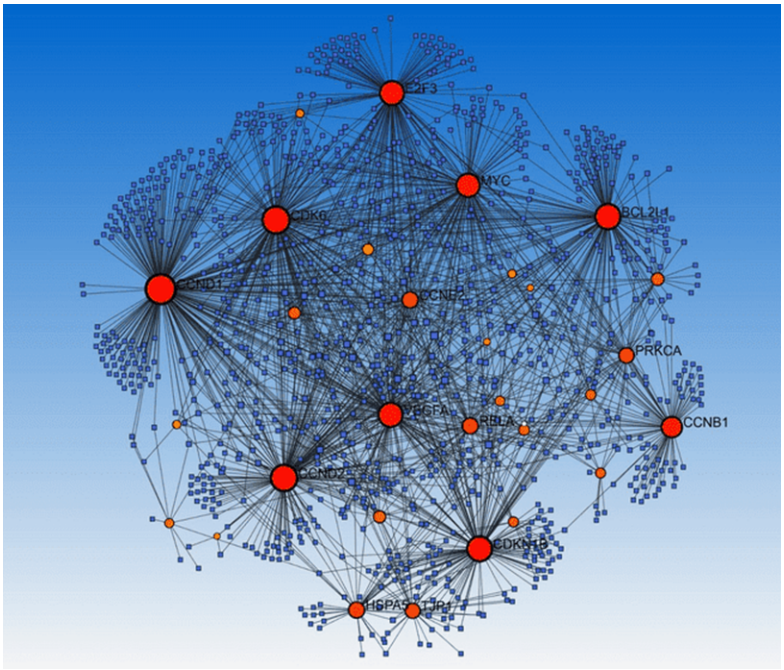

Figure 5.1: **Interactions of miRNA gene network from the last research paper.**

Hub miRNA	**Degree**	**Major Interpretation**
hsa-miR-34a-5p	11	Tumor suppressor; regulates CCND1, CDK6 and MYC-related proliferation
hsa-miR-16-5p	9	Targets cyclin-related genes and supports cell-cycle suppression
hsa-miR-335-5p	9	Associated with stemness, apoptosis and tumor plasticity regulation

Table 5.2: Major miRNA Hubs

5.3 Transcription Factor-Gene Regulatory Network

The TF-gene network analysis identified five dominant regulatory hubs: MYC with the highest degree of 79, CCND1 at 64, VEGFA at 58, RELA at 35, and CDKN1B at 25. Together, these nodes convey key molecular themes related to both

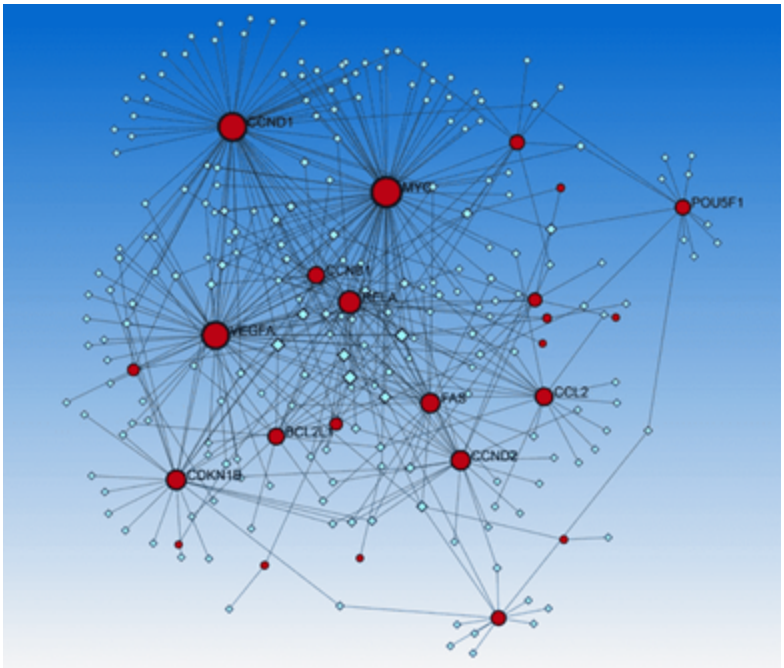

Figure 5.2: **Transcription factor-gene regulatory network from the final research paper.**

pancreas and related cancers. Signs of malignancy and inflammatory pseudocyst (proliferative drive, angiogenic). Cell-cycle checkpoint control, inflammatory survival pathways and support.

TF/Regulatory Hub	**Degree**	**Functional Meaning**
MYC	79	Global regulator of proliferation, metabolism and ribosomal biogenesis
CCND1	64	Cell-cycle progression and uncontrolled proliferation
VEGFA	58	Angiogenesis and vascular support
RELA	35	NF-kB-mediated inflammatory survival signaling
CDKN1B	25	Cell-cycle regulation and tumorigenesis-related control

Table 5.3: Major TF Hubs

5.4 Pancreas Tissue-Specific Integrated Network

This was complemented by pancreas tissue-specific interaction data for further support of the biological observations. The importance of the hub molecules of the general regulatory networks. Since can vary widely from one tissue to another, as in cases of regulatory interactions. hubs are kept central withing a pancreas-specific context, providing additional confidence to their. They give high hopes for candidacy as markers for pancreatic disease.

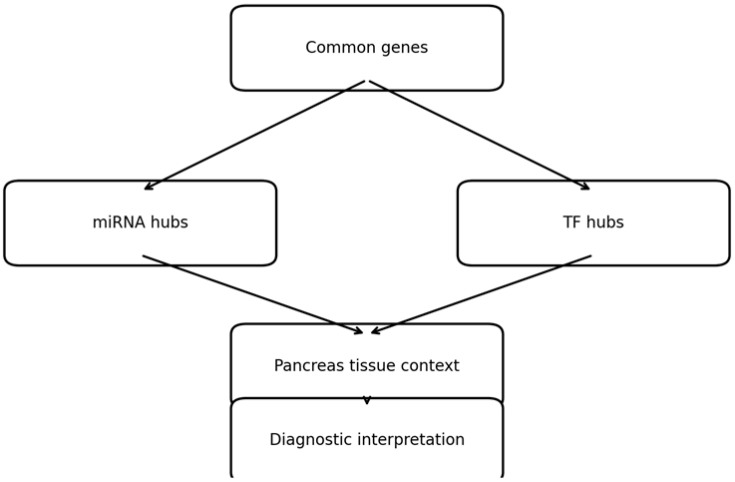

Figure 5.3: **Integrated interpretation scheme linking common genes, miRNA hubs, TF hubs and tissue context for pancreas.**

5.5 Proposed Biomarker Panel

This thesis presents an interdisciplinary approach, based on the coinciding results from the various regulatory networks, to address several questions. miRNA hubs is derived from the tumor suppressor miRNAs.The tumor suppressor miRNAs are associated with oncogenic miRNA hubs in a candidate biomarker panel. regulatory genes.The diagnostic strength of this panel would not reside in any single marker but rather in the composite expression profile across miRNA-mediated and transcriptional regulatory layers.

Category	**Candidate Biomarkers**	**Rationale**
miRNA hubs	hsa-miR-34a-5p, hsa-miR-16-5p, hsa-miR-335-5p	Tumor-suppressive regulators controlling cell-cycle, apoptosis and plasticity genes
TF/regulatory hubs	MYC, CCND1, VEGFA, RELA, CDKN1B	High-degree regulators linked with proliferation, angiogenesis, inflammation and cell-cycle control
Combined panel	miRNA hubs + TF/regulatory hubs	Potential to distinguish malignant and benign inflammatory pancreatic processes after validation

Table 5.4: Biomarker Panel

Chapter 6

Discussion

6.1 Interpretation of Shared Genes

The 31 genes shared by PDAC and pancreatic pseudocyst suggest a degree of similarity. A molecular convergence between these two conditions. This is not a reason for this observation to be read as proof that pseudocyst is malignant — but rather it simply means that it reflects the A familiar fact, that inflammation, tissue damage and cancer may involve similar biological programs. The common set of genes is thus a set of pathways that are broadly similar. significant in the remodeling of non-neoplastic tissues and tumor progression.

Now, it should be noted that these results come with certain caveats, in particular, that network centrality of a high level of: A computational model is an observation which generates hypotheses, but not a diagnosis. Nevertheless, the value of this approach lies in its ability to systematically narrow the candidate field and draw attention to biologically plausible regulators worth pursuing in experimental studies.

6.2 Biological Significance of miRNA Hubs

All three miRNA hubs identified in this study carry predominantly tumor-suppressive interpretations. If the activity of these miRNAs is attenuated in disease, their oncogenic targets — including MYC, CCND1, and CDK6 — could escape regulation and contribute to pathological proliferation. Of these, hsa-miR-34a-5p deserves particular attention given its highest degree in the miRNA-gene network and its

broad regulatory reach over multiple cell-cycle genes.

The interpretation must remain cautious because shared network centrality is a hypothesis-generating result. Nevertheless, it is valuable because it narrows the number of candidates and identifies biologically meaningful regulators for future focused validation.

6.3 Biological Significance of TF/Regulatory Hubs

The TF and regulatory gene hubs add a complementary interpretive layer to the miRNA findings. MYC and CCND1 speak to the proliferative drive of the disease, VEGFA to its vascular dependency, RELA to the co-option of inflammatory survival pathways, and CDKN1B to disruptions in cell-cycle control. As a set, these hubs trace a regulatory logic that links chronic inflammatory signaling to the conditions that support tumor progression.

The interpretation should be kept cautious as shared network centrality is a hypothesis generating result. However, it's useful as it reduces the number of candidates. and selects biologically relevant regulators for future focused validation.

6.4 Diagnostic Implications

Genetic tests is the best available method for the initial diagnosis of PDAC.A combination of several genetic tests is the most reliable method in the initial diagnosis of PDAC, and is an example of a panel. The ability to discriminate would be higher for the combination of the biomarkers than for any single biomarker. By capturing multiple aspects of the regulatory context at the same time — After replicated, miRNA acts through suppression of expression of genes that may be oncogenic, or by activation of inflammatory pathways. engagement, and angiogenic signaling — such a panel has a higher chance of reliably predicting the likelihood of a cancer's success. distinguishing the two diseases in clinical cases of similar presentation and view. inconclusive.

Uncertainty of interpretation is to be preserved since shared network centrality is a hypothesis. generating result. However, it is worth for the fact that it takes down the number of candidates. and is able to identify for future focused validation,

biologically relevant regulators.

6.5 Strengths of the Study

The main asset of this study is the merging of several analytical and layered approaches. By The disease-gene map integrates curated disease-gene information, various network types, and topological hub ranking as well as. The process of biomarker selection becomes systematic and rigorous when it is tissue-specific contextualization. and biological interpretability. The approach is also conceptually aligned with modern systems-biology thinking, which frames disease as a network phenomenon rather than a collection of individually altered genes.

The interpretation must remain cautious because shared network centrality is a hypothesis-generating result. Nevertheless, it is valuable because it narrows the number of candidates and identifies biologically meaningful regulators for future focused validation.

6.6 Limitations

The study is purely computational and rests on database-curated interaction data, which means it has not measured actual gene or miRNA expression levels in patient specimens. Diagnostic performance metrics such as ROC analysis, sensitivity, specificity, and cross-cohort reproducibility have not been evaluated. Furthermore, a high degree in a regulatory network does not automatically imply causal dominance in disease. For all these reasons, the biomarker candidates proposed here must be subjected to rigorous experimental and clinical validation before any clinical application can be considered.

The interpretation must remain cautious because shared network centrality is a hypothesis-generating result. Nevertheless, it is valuable because it narrows the number of candidates and identifies biologically meaningful regulators for future focused validation.

6.7 Validation Strategy

Experimental validation of these findings should involve quantitative RT-PCR profiling of the candidate miRNAs and their target mRNAs in PDAC tissue, pseudocyst samples, and matched healthy controls. Plasma or serum-based circulating miRNA analysis could serve as a non-invasive diagnostic avenue. Statistical modeling approaches should then be applied to determine whether the combined biomarker panel meaningfully outperforms individual markers in distinguishing the two conditions.

The interpretation must remain cautious because shared network centrality is a hypothesis-generating result. Nevertheless, it is valuable because it narrows the number of candidates and identifies biologically meaningful regulators for future focused validation.

6.8 Clinical Translation

This need the panel to prove robustness, repeatability of results from different laboratories, reasonable cost, and the ability to interpret the results, Along with normal imaging results. It would also have to be tested in well. There is need to characterize patient cohorts with confirmed diagnosis by histopathology and/or other "gold standard" methods. The overall clinical goal would be to minimize the number of diagnoses. ambiguity on pancreatic lesion evaluation and more prompt and accurate clinical diagnosis. management.

The interpretation should be kept on hold since shared network centrality is a hypothesis generating result. Nevertheless, it is good to have it as it reduces the number of candidates. and provides biologically relevant regulators to be targeted for future focused validation.

Limitation	Impact	Suggested Solution
Database dependency	May include context-independent interactions	Validate in pancreatic tissue and disease-specific cohorts
No experimental expression data	Cannot confirm upregulation or downregulation in samples	Perform qRT-PCR or RNA sequencing
No clinical performance metrics	Diagnostic utility remains unknown	Use ROC analysis, sensitivity and specificity testing
Hub degree is not causality	High connectivity may not equal functional dominance	Use functional assays and knockdown/overexpression studies

Table 6.1: Limitations and Validation Requirements

Chapter 7

CONCLUSION AND FUTURE SCOPE

This chapter is the sum and substance of the entire thesis and also outlines future directions for experimental testing and clinical translation.

7.1 Conclusion

This thesis provides a systems biology study on regulatory network-driven discovery of biomarkers for the discrimination of PENC from PCA. pseudocyst. The original research paper was elaborated to a full-fledged academic thesis. Following the DTU front matter requirements, chapter structure and formatting standards. Using the intersection analysis of these two data sets and gene retrieval via CTD, 31 genes shared between PDAC and their orthologs in C. albicans were identified. Two biological processes were identified and were pancreatic pseudocyst. These mechanisms encompass the control of cell cycle, apoptosis, inflammatory signals and angiogenesis.

The interaction analysis of the hubs unraveled by Network construction on NetworkAnalyst gave rise to three hubs of miRNAs: hsa-miR-34a The top five TF/regulatory hubs are: The genes for MYC, CCND1, VEGFA, RELA and CDKN1B. A tissue-specific network analysis is performed.The tissue-specific network analysis is done. These regulators were confirmed by confirmed to still be relevant in the pancreatic context. Taken together, the data confirm the idea that a diagnostic panel can be developed based on tumor-suppressive miRNA hubs. and

oncogenic gene hubs might become a basis for further differentiation of PDAC from pancreatic pseudocyst.

Overall, this thesis states that a systems-biology approach is meaningful, benefits for prioritization of biomarkers with capture of molecular relationships instead of Isolating genes to work with. The results reported below are computational, however, and is to be considered as a hypothesis generation in nature, not a clinical ground truth. Experimental These will need to be confirmed in biological samples and stringent clinical testing prior to their use. The proposed biomarkers can be used in the clinics.

7.2 Future Scope

Future studies should aim to confirm the proposed biomarker candidates in the following: Patient derived samples such as pancreatic tissue, pseudocyst fluid, and blood-based (serum and plasma) samples. The hsa-miR-34a-5p and hsa-miR-16-5p expression analysis was introduced. The expression analysis of hsa-miR-34a-5p and hsa-miR-16-5p was introduced. hsa-miR-335-5p and MYC, CCND1, VEGFA, RELA and CDKN1B must be performed with. By qRT-PCR, RNA sequencing or other molecular techniques. Sufficiently large and accuracy.To obtain reliable estimates of diagnostic accuracy, independent patient cohorts will be required. accuracy.

The machine learning classifiers that were trained using the biomarker panel may be utilized to: The discrimination between PDAC and pancreatic pseudocyst should be automated, and these should be monitored. The distinction between PDAC and pancreatic pseudocyst should be automated and pseudocysts should be monitored. evaluated rigorously with sensitivity and specificity, area under the ROC curve and independent external validation. Addition of further molecular dimensions such as proteomic Additional structural features such as profiles, data from DNA methylation patterns and from single cells — could further enrich the information available for each individual. biological resolution. The network-based panel that is proposed in this thesis could eventually, these lead to better and more efficient diagnostic processes, especially if In addition to clinical evaluation and imaging modalities.

Chapter 8

CHAPTER-WISE EXTENDED SUMMARY

8.1 Introduction summary

The introduction explains the clinical challenge created by the similarity between PDAC and pancreatic pseudocyst. It also establishes why regulatory network analysis is suitable for identifying candidate biomarkers in complex pancreatic conditions.

This section was included to help readers follow the logical progression from clinical problem to computational analysis, results, interpretation and future work.

8.2 Literature review summary

The literature review describes the key biological layers relevant to the study: pancreatic cancer biology, inflammatory pseudocyst formation, miRNA-mediated regulation, transcription factor hubs and systems-biology biomarker discovery.

This section was included to help readers follow the logical progression from clinical problem to computational analysis, results, interpretation and future work.

8.3 Methods summary

The methods chapter describes retrieval of disease-associated genes, intersection analysis to identify common genes, NetworkAnalyst-based construction of regu-

latory networks and hub prioritization using degree centrality.

This section was included to help readers follow the logical progression from clinical problem to computational analysis, results, interpretation and future work.

8.4 Results summary

The results chapter reports 31 common genes, three major miRNA hubs and five major TF/regulatory hubs. It also explains the pancreas tissue-specific network and proposes a combined biomarker panel.

This section was included to help readers follow the logical progression from clinical problem to computational analysis, results, interpretation and future work.

8.5 Discussion summary

The discussion chapter interprets the biological relevance of shared genes and regulatory hubs. It emphasizes that the findings are computational and require validation before clinical application.

This section was included to help readers follow the logical progression from clinical problem to computational analysis, results, interpretation and future work.

8.6 Conclusion summary

The Causes of Biodiversity (CAB) group summarized that a network-driven perspective is able to highlight biologically meaningful Future studies are needed to establish which are the best biomarker candidates to distinguish PDAC from pancreatic pseudocyst. It is crucial to have experimental validation. This section was added to aid via the logical flow of the material from clinical problem to computational analysis, results, interpretation and future work.

Appendix A

COMMON GENE AND HUB LIST

A list of key molecular entities discussed is summarized below in the following Appendix. throughout this thesis. The computational procedure used to identify them should be 'trial begins.tested with experimental expression data and patient cohorts before clinical trials. application is attempted.

Entity Type	**Molecules**	**Interpretation**
Common genes	31 overlapping genes from CTD	Shared molecular background between PDAC and pancreatic pseudocyst
miRNA hubs	hsa-miR-34a-5p, hsa-miR-16-5p, hsa-miR-335-5p	Tumor-suppressive regulatory candidates
TF/regulatory hubs	MYC, CCND1, VEGFA, RELA, CDKN1B	High-connectivity nodes involved in proliferation, angiogenesis, inflammation and cell-cycle control

Table A.1: Common Genes And Hubs Summary

Appendix B

SAMPLE VALIDATION PLAN

- Under ethical guidelines, collection of confirmed PDAC, pancreatic pseudocyst and control samples. approval.
- Use qRT-PCR or sequencing to measure the expression of the candidate miRNAs and hub genes.
- Statistically assess diagnostic performance of single and combined tests.
- Validate the model in an independent cohort.
- Compare biomarker results with imaging and clinical parameters.

Appendix C

EXTENDED BACKGROUND NOTES

C.1 Right to early diagnostic discrimination and access to treatment

An early distinction between malignant and benign pancreatic lesions can be of great importance. have clinical significance as both illnesses require completely different treatment. strategies. Oncological workup, staging and treatment planning are required in a timely manner for PDAC, and While pancreatic pseudocyst is dealt with by size, symptom severity, infection risk, and depending on the underlying severity of pancreatitis. If clinical and radiological characteristics of These two conditions are shared, and a molecular panel could give an additional and more detailed analysis of the molecular basis than any one of them alone.

Objective layer of evidence to inform clinical practice decisions. It is proposed to create a network-based panel in: The thesis used for this purpose should thus be interpreted as a tool for decision making and not as a replacement. For established diagnostic modalities, as well as for subjective evaluations of test results. However, in any subsequent clinical use, such molecular markers would have to be tested. In conjunction with conventional radiologic and biochemical markers and not as a replacement. The use of molecular biomarkers in conjunction with imaging data, may improve Provide diagnostic clarity and eliminate ambiguity in ambiguous cases, especially — which is particularly urgent In pancreatic disease, diagnosis is a critical issue to consider because an early diagnosis is significant in terms of

patient outcomes.

C.2 Inflammatory overlap in pancreatic lesions

Molecules can be secreted in inflammation processes that are very similar to those produced in cancer. What are the consequences of a cytokine cascade, immune cell infiltration, hypoxia and tissue repair? The expression profiles are all affected by the mechanisms, by matrix remodeling. This Offers a plausible explanation for the genetic similarity of an inflammatory condition As in pseudocyst and a tumour, such as PDAC. The presence of shared genes should not be Interpreted as indications of malignancy — no, actually, it is a sign of both types of growth. stages and have them complement the specific characteristics of each disease.It is necessary to leverage shared biological programs as needed in each of their disease states while complementing the disease-specific characteristics. The pancreatic microenvironment is a setting of several courses.

These markers should be studied with radiological and biochemical indicators. Combining molecular biomarkers with imaging can increase the amount of interpretability and decrease uncertainty in ”borderline” cases. This is particularly important: for pancreatic diseases with a significant treatment impact due to delayed diagnosis.

C.3 Value of miRNA biomarkers

There are several reasons that miRNAs are good biomarker candidates: each miRNA can simultaneously control multiple target genes and consequently pathway level control. Both and miRNAs are detectable in tissue and other biological fluids like blood. A Thus, a small set of miRNAs can reflect intricate regulation states, which are very comparable to the one associated with a single gene. Some error may be possible in the number of mRNA markers. In this thesis, the candidates hsa-miR-34a-5p, hsa-miR-16-5p and hsa-miR-15a-5p were chosen. To choose the priority candidates, a subset of microRNAs with high centrality in the miRNAs-mRNA regulatory network was selected as hsa-miR-335-5p. regulation of gene expression in humans and other organisms.Known miRNA-gene regulatory network and their roles in biological processes.

For future clinical workflow these markers are to be used alongside radiological and biochemical indicators. The use of molecular biomarkers in combination with imaging can enhance. Is interpretable and can alleviate subjectivity in ambiguous situations. This is of particular significance, for pancreatic diseases which can have a significant impact on outcome if diagnosed late

C.4 Need for combined panels

No single biomarker will likely be adequate to distinguish PDAC from pseudocyst because they have been found in These shared inflammatory and tissue remodeling signatures included. A strong diagnostic approach is, therefore, required to make an accurate diagnosis. needs a panel, which allows to measure several aspects of the molecular nature of the disease. This is accomplished by the panel presented in this thesis of tumor-suppressive miRNA hubs. With high degree regulatory genes, these combine to cover the proliferative, apoptotic, Inflammatory and angiogenic aspects of the biology of both diseases.

Appendix D

CANDIDATE BIOMARKER

D.1 hsa-miR-34a-5p

This miRNA was the highest-degree miRNA hub in the regulatory network. It is interpreted as a tumor-suppressive regulator and is associated with inhibition of cell-cycle progression. In the research paper, it was linked to regulation of CCND1, CDK6 and MYC. Its diagnostic relevance arises from its ability to represent a broad anti-proliferative regulatory program.

Recommended validation: measure expression in confirmed PDAC samples, pseudocyst-associated samples and non-diseased controls. Evaluate whether the candidate improves classification alone and in combination with other markers. Compare the molecular result with imaging, histopathology and clinical diagnosis wherever available.

D.2 hsa-miR-16-5p

This miRNA was identified as a high-degree hub with a degree value of 9. It is associated with control of cyclin-related genes and G1/S transition. In the context of PDAC and pseudocyst, altered miR-16-5p activity may indicate abnormal cell-cycle activation shared by inflammatory and neoplastic pancreatic changes.

Recommended validation: measure expression in confirmed PDAC samples, pseudocyst-associated samples and non-diseased controls. Evaluate whether the candidate improves classification alone and in combination with other markers.

Compare the molecular result with imaging, histopathology and clinical diagnosis wherever available.

D.3 hsa-miR-335-5p

This miRNA was also identified as a high-degree hub with a degree value of 9. It is linked with genes associated with stemness, apoptosis and cell plasticity. The presence of some of the items, including its addition to the panel, is important as tumour progression is not just about proliferation but also survival, invasiveness and adaptive states of the cell.

Recommended validation: measure expression in confirmed PDAC samples, pseudocyst-associated samples and non-diseased controls. Evaluate whether the candidate improves classification alone and in combination with other markers. Compare the molecular result with imaging, histopathology and clinical diagnosis wherever available.

D.4 MYC

MYC showed the highest degree among the major regulatory hubs. It is a global regulator of proliferation, metabolism and biosynthetic activity. Its high degree indicates broad network connectivity. In diagnostic interpretation, MYC can represent the proliferative and metabolic dimension of malignant biology.

Recommended validation: measure expression in confirmed PDAC samples, pseudocyst-associated samples and non-diseased controls. Evaluate whether the candidate improves classification alone and in combination with other markers. Compare the molecular result with imaging, histopathology and clinical diagnosis wherever available.

D.5 CCND1

CCND1 showed a high degree value and is directly associated with cell-cycle progression. It is particularly relevant because it is also discussed as a target of tumor-suppressive miRNAs. The relationship between CCND1 and miRNA hubs supports the value of studying regulatory pairs rather than isolated genes.

Recommended validation: measure expression in confirmed PDAC samples, pseudocyst-associated samples and non-diseased controls. Evaluate whether the candidate improves classification alone and in combination with other markers. Compare the molecular result with imaging, histopathology and clinical diagnosis wherever available.

D.6 VEGFA

VEGFA is a major angiogenic regulator. Its presence as a hub indicates the importance of vascular and hypoxia-associated processes in pancreatic disease biology. In PDAC, angiogenic signaling can support tumor growth, while inflammatory lesions may also involve vascular remodeling.

Recommended validation: measure expression in confirmed PDAC samples, pseudocyst-associated samples and non-diseased controls. Evaluate whether the candidate improves classification alone and in combination with other markers. Compare the molecular result with imaging, histopathology and clinical diagnosis wherever available.

D.7 RELA

RELA is a part of the NF-kB signaling pathway and links inflammation with survival. pathways. Its inclusion is a biologically relevant one as pseudocyst is inflammatory while PDAC can use inflammatory signaling to make progress. Therefore, RELA might be an indicator of Act as a link between non-cancerous inflammation and cancerous survival signals. Validated: Yes (in PDAC samples) Likely associated samples as well as their respective non-diseased controls. Assess candidate's improvement As a standalone or along with other markers. To compare the molecular result Accompanied by imaging, histopathology and clinical diagnosis, as available.

D.8 CDKN1B

CDKN1B, which is associated with the cell cycle, was found to be in a hub position in the TF-gene network. It is used, sometimes in more than one way, but in so many ways as to suggest its importance in the network. Shared pancreatic disease

signatures associated with of cell-cycle control. Worst case scenario: Confirm in samples from PDAC, pseudocyst Associated samples and non diseased controls. Examine and assess if the candidate enhances On its own, and in combination with other markers. Compare the molecular result to the given molecular equation. Imaging and histopathology, wherever available, with clinical diagnosis.

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