USING GENE-miRNA NETWORKS ANALYSIS TO IDENTIFY POSSIBILITIES IN CURRENTLY AVAILABLE MEDICATIONS FOR CHRONIC HEPATITIS-INDUCED HCC

Thesis submitted
in partial fulfilment of the requirements for the
degree of

MASTER OF SCIENCE
in
BIOTECHNOLOGY

by

KAUSHIKI
23/MSCBIO/25

Under the supervision of

DR. ASMITA DAS
Associate professor
Department of Biotechnology

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, New Delhi, 110042

DECLARATION

I, Kaushiki 23/MSCBIO/25 hereby certify that the work which is being presented in the thesis entitled “Using Gene-miRNA Networks analysis to identify possibilities in currently available medications for Chronic Hepatitis-Induced HCC” 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 2023 to 2025 under the supervision of Dr. Asmita das (Associate Professor).

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Candidate's Signature

SUPERVISOR'S CERTIFICATE

Certified that Kaushiki (23/MSCBIO/25) has carried out their search work presented in this thesis entitled "Using Gene-miRNA Networks analysis to identify possibilities in currently available medications for Chronic Hepatitis-Induced HCC" for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Date:

Signature

Dr. Asmita das

Associate Professor

Department of Biotechnology

Delhi Technological University

Signature

Prof. Yasha Hasija

Head of the Department

Department of Biotechnology

Delhi Technological University

Abstract

This report presents a detailed bioinformatics investigation into the molecular underpinnings of chronic hepatitis-induced Hepatocellular Carcinoma (HCC), aiming to identify significantly differentially expressed genes (DEGs) and microRNAs (miRNAs) and to pinpoint therapeutic drugs that can modulate these dysregulated networks. Utilizing public gene expression datasets (e.g., GSE121248) and bioinformatics tools, the analysis revealed a distinct molecular landscape critical to HCC progression.

The study identified a comprehensive list of the 10 most important downregulated genes, including key tumor suppressors like CDHR2, ACADS, and IGFBP3, alongside the 10 most important upregulated genes, such as SCN3B, OGG1, and KNOP1, many of which are associated with oncogenic processes.

Furthermore, the report details the mechanisms of action and miRNA-modulating capabilities of several therapeutic agents. Cisplatin, a DNA-damaging chemotherapy, upregulates the tumor-suppressor hsa-miR-34a, enhancing its cytotoxic effects. Sorafenib, a multi-kinase inhibitor, demonstrates a dual miRNA-modulating role by upregulating tumor-suppressive let-7 family members while downregulating the oncogenic hsa-miR-21-5p. In contrast, Regorafenib and Everolimus, while highly effective kinase and mTOR inhibitors respectively, do not directly target miRNAs but indirectly influence the gene-miRNA network by impacting genes that are themselves subject to miRNA regulation.

This comprehensive analysis highlights the complex interplay within gene-miRNA networks in HCC pathogenesis and provides a mechanistic basis for how existing drugs influence these networks, offering insights for potentially enhancing therapeutic efficacy and overcoming resistance in HCC treatment.

Keywords:
Hepatocellular Carcinoma (HCC), Chronic Hepatitis, Gene-miRNA Network, Differential Gene Expression, MicroRNAs (miRNAs), Sorafenib; Drug Resistance; Bioinformatics, Targeted Therapy; Network Pharmacology

ACKNOWLEDGEMENT

First of all, I would like to extend my heartfelt gratitude to my supervisor, Dr.. Asmita Das, for her constant support and encouragement throughout the course of my research. Her expertise and constant constructive feedback have always been invaluable to me in learning and understanding new yet difficult things. She has always been an inspiration to me in pursuing my research and future goals. I would also like to thank Ms. Simran , Mr. Yuvraj, Ms. Sweta gulia and each and every member of the Immunology laboratory for always helping and guiding me through each up and down which has come along the way and giving me an environment where I can learn and grow as a person.

I am also sincerely grateful to the faculty especially professors of the Department of Biotechnology, Delhi Technological University for providing me an academic environment which is coupled with theoretical as well as practical aspects of academia and providing me every necessary help and resource to carry out my work. A special thanks to Mr. Jitender Singh, Mr. C.B. Singh, Mr. Lalit, Mr. Jaspreet, and Mr. Rajesh for their technical assistance and guidance through the project.

Finally, I would like to whole-heartedly thank my family and friends for always being my constant support and well-wishers. Their presence and belief in me have always been a source of motivation and strength, their contribution in my life can't be put into words.

Thank you all for your valuable contributions and sacrifices which made this project a success.

Kaushiki 23/MSCBIO/25

Table of Contents

List of Figures

List of Tables

Table 1: Comprehensive List of Abbreviations from 'HCC Gene, miRNA, Drug Analysis' Thesis

Abbreviation	Full Form
3'-UTRs	3'-untranslated regions
ABC	ATP-binding cassette
BER	Base Excision Repair
Bcl-xL	B-cell lymphoma-extra large
CCND1	Cyclin D1
CDC25A	Cell Division Cycle 25A
CLIP-seq	Cross-Linking Immunoprecipitation with sequencing
CYP3A4	Cytochrome P450 3A4
DEGs	Differentially Expressed Genes
EMT	Epithelial-Mesenchymal Transition
ERK	Extracellular signal-regulated kinase
FGFR	Fibroblast Growth Factor Receptor
FLT-3	Fms-like Tyrosine Kinase 3

GEO	Gene Expression Omnibus
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HMGA2	High Mobility Group AT-hook 2
KIT	KIT proto-oncogene receptor tyrosine kinase
MAPK	Mitogen-Activated Protein Kinase
MEK	Mitogen-activated protein kinase kinase
MET	MET proto-oncogene, receptor tyrosine kinase
miRNA	MicroRNA (also appears as miRNAs, plural form)
mRNAs	messenger RNAs
MTIs	miRNA-target Interactions
MYC	Myelocytomatosis oncogene
NASH	Nonalcoholic Steatohepatitis

NCBI	National Center for Biotechnology Information
oncomiRs	oncogenes (miRNAs acting as oncogenes)
PD-L1	Programmed Death-Ligand 1
PDAC	Pancreatic Ductal Adenocarcinoma
PDGFR	Platelet-Derived Growth Factor Receptor
PgP	P-glycoprotein
PPI	Protein-Protein Interaction
RAS	Rat Sarcoma virus oncogene
RET	RET proto-oncogene
ROS	Reactive Oxygen Species
TIE2	Tyrosine Kinase with Immunoglobulin-like and EGF-like Domains 2
TKIs	Tyrosine Kinase Inhibitors
TME	Tumor Microenvironment
TNM	Tumor, Node, Metastasis

Chapter.1

Introduction: Hepatocellular Carcinoma (HCC) and the Rationale for Gene-miRNA Network Analysis

1.1 Global Burden and Etiology of HCC, Particularly Chronic Hepatitis-Induced HCC

Hepatocellular carcinoma (HCC) stands as a formidable and escalating global health crisis, consistently ranking among the most prevalent and lethal malignancies worldwide. Its significant impact on public health is underscored by its position as the sixth most common cancer globally, with an estimated 866,136 new cases reported in 2022 (Sung et al., 2021). The disease disproportionately affects men, where it is the fifth most common cancer, compared to women, where it ranks ninth. The sheer scale of its lethality is evidenced by the 758,725 deaths attributed to HCC globally in 2022, highlighting its exceptionally high mortality rate. This makes HCC a leading cause of cancer-related mortality, accounting for approximately 80% of all primary liver cancers (Sung et al., 2021). The global incidence of HCC has shown a concerning upward trend, with a substantial 70% increase in cases worldwide since 1990 (Sung et al., 2021).

Geographically, viral hepatitis continues to be the predominant cause of HCC in many regions, particularly across Asia and most of Africa. However, the epidemiological landscape of HCC is undergoing a profound transformation. A notable shift in the global burden of the disease is observed, moving from regions with low-to-moderate sociodemographic indices to those with higher indices. This demographic transition reflects a corresponding shift in primary etiologies, from predominantly viral infections to non-viral causes (Yang et al., 2023).

The evolving epidemiology, particularly the increasing prevalence of NASH-related HCC, signifies that future therapeutic strategies must consider a broader spectrum of molecular drivers beyond those exclusively associated with viral hepatitis. This dynamic and expanding challenge, characterized by high and rising incidence, persistent high mortality, and a changing etiological profile, underscores the urgent and persistent unmet need for innovative and effective treatments.

1.2 Molecular Pathogenesis of HCC:

Role of Gene Expression Deregulation and MicroRNAs (miRNAs)

The initiation, progression, and metastatic dissemination of HCC are fundamentally driven by an intricate and profoundly dysregulated molecular pathogenesis (Heimbach et al., 2024). This complex process is characterized by extensive alterations in gene expression patterns, with gene expression profiling consistently revealing a large number of differentially expressed genes (DEGs) that are directly implicated in these oncogenic events. These DEGs play critical roles in various cellular processes, including uncontrolled cell proliferation, evasion of apoptosis, and enhanced metastatic potential.

MicroRNAs (miRNAs), a family of tiny, non-coding RNAs that serve as essential post-transcriptional regulators of gene expression, are at the heart of this molecular dysregulation (Lee et al., 2024). One By attaching to complementary sequences, mostly found in the 3'-untranslated regions (3'-UTRs) of target messenger RNAs (mRNAs), miRNAs carry out their regulatory actions. In order to fine-tune gene expression, this binding usually results in either the destruction of the target mRNA or the blockage of mRNA translation into protein. It is often seen that miRNAs exhibit abnormal expression in HCC. Their dysregulation significantly impacts key oncogenic and tumor-suppressor pathways, functioning as either tumor suppressors or oncogenes (oncomiRs). Despite the growing understanding of individual DEGs and miRNAs, a significant gap persists in the comprehensive elucidation of the intricate gene-miRNA regulatory networks, particularly within the context of drug-resistant chronic hepatitis-induced HCC (Lee et al., 2024). This knowledge deficit extends to understanding the full potential of targeting these complex networks with therapeutic agents. A particularly novel and underexplored area is the identification of drugs capable of bidirectionally modulating miRNA expression—meaning, simultaneously upregulating beneficial tumor-suppressor miRNAs while downregulating detrimental oncogenic miRNAs.

The inherent complexity of HCC pathogenesis, driven by interconnected dysregulation involving both genes and miRNAs, suggests that traditional single-target therapies may be insufficient for achieving durable responses. The intricate web of interactions means that altering one component can trigger compensatory mechanisms elsewhere in the network, leading to treatment failure. Therefore, a multi-pronged approach that can precisely modulate the expression of both oncogenic and tumor-suppressor miRNAs within the regulatory network is increasingly recognized as a necessary strategy for effective and lasting HCC treatment.

1.3 Current Therapeutic Challenges and the Unmet Need for Novel Strategies

Despite significant advancements in cancer therapy, the prognosis for patients with advanced HCC remains notably poor. A primary contributing factor to this grim outlook is the almost inevitable development of drug resistance, which severely limits the long-term efficacy of available treatments.About 15-20% of people with HCC are candidates for possibly curative procedures including liver transplantation or surgical resection. As a result, the great majority of patients are dependent on systemic treatments, which are often hindered by resistance even though they provide some advantage in terms of survival.

For over a decade, multi-targeted tyrosine kinase inhibitors (TKIs) like Sorafenib dominated the systemic treatment landscape for HCC. Sorafenib, approved in 2007, was the sole systemic agent for advanced HCC until 2016, (Wilhelm et al., 2004) extending the median overall survival from a mere 8 months to 11 months. Subsequently, Lenvatinib and Regorafenib gained approval as first- and second-line treatments, respectively, further expanding the therapeutic arsenal (Llovet et al., 2018). These TKIs primarily exert their anti-tumor effects by inhibiting key kinases involved in tumor angiogenesis and proliferation.

The first-line treatment for unresectable HCC has seen a dramatic paradigm shift in recent years, shifting toward immunotherapy-based regimens. When compared to Sorafenib, the combination of bevacizumab (an anti-VEGF-A monoclonal antibody) and atezolizumab (an anti-PD-L1 monoclonal antibody) has shown improved overall survival (Finn et al., 2020), radically changing the standard of therapy. However, even with these immunotherapeutic breakthroughs, a substantial clinical need for further optimization persists, as less than one-third of patients achieve an objective response (Finn et al., 2020).

The pervasive issue of drug resistance in HCC manifests in two primary forms: primary resistance, where tumor cells are inherently insensitive to the drug from the outset due to genetic heterogeneity, and acquired resistance, which develops after an initial period of clinical benefit, rendering previously effective therapies ineffectiveTKI resistance is caused by incredibly intricate and varied processes. These include the activation of bypass signaling pathways (such as the PI3K/mTOR, RAS/RAF/MEK/ERK, and EGFR pathways) (Wang et al., 2023), which enable cancer cells to continue to proliferate and survive even when their primary targets are inhibited by drugs. . The overexpression of ATP-binding cassette (ABC) transporters, which actively pump drugs out of cancer cells, the epithelial-mesenchymal transition (EMT), which increases cell motility and invasiveness, and hypoxia adaptations in the tumor microenvironment (TME) are additional contributing factors.. Moreover, metabolic reprogramming, changes in programmed cell death pathways (like autophagy and ferroptosis), and the appearance of "persister" cells—cells with non-genetic modifications that allow them to withstand drug

exposure and aid in tumor recurrence—all significantly contribute to the development of resistance.

Specific pathways implicated in Sorafenib resistance include the Hippo-YAP signaling axis, which can activate SLC7A11 to increase intracellular glutathione levels and inhibit reactive oxygen species, thereby protecting cancer cells from drug-induced oxidative stress (Feng et al., 2023). Other mechanisms involve LCN2, NF-κB, and the Keap1-Nrf2 system, alongside broader epigenetic regulation (Yang et al., 2024), altered programmed cell death, and metabolic reprogramming. For Lenvatinib resistance, dysfunctional pathway activation, altered drug transport, and specific regulated cell death mechanisms (including apoptosis, autophagy, and ferroptosis) are implicated, often influenced by cancer stem cells.

The complex and pervasive nature of drug resistance in HCC underscores a fundamental limitation of single-target therapies. When a single pathway is inhibited, cancer cells often adapt by activating alternative or compensatory pathways, leading to treatment failure. This continuous cycle of resistance necessitates a paradigm shift towards therapeutic strategies that can simultaneously target multiple nodes within the intricate molecular networks driving cancer progression and resistance. A gene-miRNA network analysis is uniquely positioned to identify such multi-target interventions. By mapping the interconnectedness of dysregulated genes and miRNAs, this approach can uncover vulnerabilities that, when targeted in combination, may offer a more robust and durable therapeutic response, ultimately improving outcomes for HCC patients. This holistic perspective is crucial for developing effective strategies to circumvent the multifaceted challenges posed by drug resistance in HCC.

Chapter 4

Materials and Methods:

2.1. Data Acquisition and Differential Gene Expression Analysis

The foundation of this comprehensive investigation into the molecular pathogenesis of chronic hepatitis-induced HCC was built upon a meticulously curated dataset obtained from publicly accessible resources. This approach ensures transparency and reproducibility, allowing other researchers to validate and expand upon the findings.

2.1.1 Data Source

The Gene Expression Omnibus (GEO) database, a well-known global public repository for functional genomics data, served as the study's main source of gene expression data. Specifically, the GSE121248 accession was utilized.[1] This particular dataset was selected for its relevance to HCC, as it contained gene expression profiles derived from 37 human liver tissue samples. Crucially, this collection included both normal liver tissues and malignant hepatocellular carcinoma tissues, along with their corresponding sample counts. The inclusion of both normal and diseased samples provided an essential comparative basis, enabling the identification of molecular alterations specific to HCC development. The use of a standardized, publicly available dataset like GSE121248 ensures that the initial data foundation is robust and verifiable, a critical aspect of rigorous scientific inquiry.

2.1.2 GEO2R-Based Differential Gene Expression Analysis

GEO2R was used as the main analysis software in order to methodically find genes that showed notable variations in expression levels between HCC and normal liver tissues. One The National Center for Biotechnology Information (NCBI) offers GEO2R, an easy-to-use web-based application that enables users to compare gene expression data within GEO series datasets. 16 Its utility lies in its ability to quickly identify differentially expressed genes (DEGs) by applying statistical methods to compare two or more defined groups of samples.

The analytical process within GEO2R involved several key, sequential steps to ensure the reliability of the identified DEGs:

Dataset Access and Loading: The GSE121248 dataset was directly accessed through the GEO platform, and its expression profiles were loaded into the GEO2R interface.

Group Definition: The 37 tissue samples within the dataset were precisely categorized into two distinct experimental groups: "normal liver tissues" and "malignant HCC tissues." This clear group definition is fundamental for accurate comparative analysis.

Data Distribution Assessment: A critical preliminary step involved assessing the value distribution of gene expression for each individual sample. This was performed by visualizing the data using box plots within GEO2R. The purpose of this step is to ensure that the gene expression distributions across all samples are median-centered and comparable. This quality control measure is a prerequisite for reliable statistical analysis, as significant deviations in data distribution between groups could introduce bias into the differential expression results. The consistency observed in the box plots confirmed the suitability of the data for comparative analysis.

Statistical Calculation: GEO2R employs robust statistical algorithms to identify genes whose expression levels are significantly altered between the defined groups. The primary metric for ranking genes is their statistical significance, typically represented by P-values. Genes with lower P-values indicate a higher statistical probability of true differential expression.

Output Generation and Export: The output of the GEO2R analysis was a comprehensive table listing all genes, sorted by their statistical significance. This table included various metrics, such as fold change and P-values, which are essential for interpreting the magnitude and reliability of gene expression changes. The results table could be customized to include specific columns (e.g., t-statistic, B-value, Gene Ontology Function annotation) and was exported as a tab-delimited file for further downstream computational and biological analyses. Furthermore, GEO2R provides access to the underlying R script used for the analysis, which significantly enhances the transparency and reproducibility of the initial gene selection process. This feature allows other researchers to independently verify the analytical steps and replicate the findings, thereby strengthening the credibility and generalizability of the identified DEGs.

2.1.3 Selection of Significant Genes

Following the rigorous differential gene expression analysis performed by GEO2R, a focused selection of genes was made for subsequent network construction. This selection was based on stringent statistical criteria, specifically their p-values and log2 fold change values.[1] To ensure that only the most robust and biologically impactful changes in gene expression were carried forward for deeper investigation, the ten genes that are most significantly up-regulated and the ten genes that are most significantly down-regulated were chosen from the comprehensive list of DEGs. This targeted approach allowed the study to concentrate on the molecular players most profoundly altered in HCC, forming a reliable and verifiable foundation for all subsequent network analysis and drug targeting efforts. The meticulous data selection and reproducible analytical methodology employed at this stage are paramount for building a credible scientific narrative and for enabling future research to build upon these findings.

2.2 Construction of Gene-miRNA Regulatory Networks

Understanding the intricate interplay between dysregulated genes and microRNAs (miRNAs) is crucial for deciphering the complex molecular pathogenesis of HCC. This study employed a multi-faceted approach to construct and visualize these regulatory networks, integrating data from multiple reputable bioinformatics databases to enhance the reliability of predicted interactions.

2.2.1 Gene-miRNA Interaction Prediction

Investigating their interactions with miRNAs was based on the top 10 genes that were highly up-regulated and the top 10 genes that were significantly down-regulated, as determined by the GEO2R study. The NetworkAnalyst database (https://www.networkanalyst.ca/) was used to complete this important stage. One An advanced web-based tool called NetworkAnalyst was created especially for visual exploration and integrative protein-protein interaction (PPI) network analysis. It is the perfect tool for this study because of its ability to process and analyze a wide range of input data, including gene/protein lists and gene expression databases.

A key strength of NetworkAnalyst lies in its ability to integrate information from multiple, high-quality curated databases for gene-miRNA interactions. This multi-source validation approach significantly enhances the confidence and biological relevance of the predicted interactions. The primary databases integrated by NetworkAnalyst for miRNA-target predictions include:

- **miRDB:** This database leverages advanced machine learning algorithms, incorporating data from miRNA overexpression experiments and CLIP-seq (Cross-Linking Immunoprecipitation with sequencing) studies, to predict miRNA targets across a wide range of species. Its computational approach provides a broad scope of potential interactions [18].

- **miRTarBase:** This is a well-known, extensive database that carefully gathers and selects miRNA-target interactions (MTIs) that have been verified by experiment. A variety of experimental methods, including Western blotting, microarrays, reporter gene assays, and next-generation sequencing studies, are used to rigorously validate these interactions. 18 A high degree of confidence in the correctness of the discovered interactions is provided by miRTarBase's emphasis on experimental validation.
- **TargetScan:** This tool predicts MTIs primarily based on the principle of seed region compatibility between the target mRNA's 3'-UTR and the miRNA, coupled with evolutionary conservation across species. TargetScan provides valuable insights into both highly conserved and less conserved target sites, offering a phylogenetic perspective on miRNA regulation.

By integrating data from these diverse databases, NetworkAnalyst ensures that the predicted gene-miRNA interactions are not reliant on a single computational model or experimental approach. This cross-validation process significantly increases the reliability and biological relevance of the constructed networks. Furthermore, NetworkAnalyst's capability to perform functional enrichment analysis and topological analysis (e.g., identifying hub nodes and modules) allows for a deeper understanding of the network's architecture and the identification of critical regulatory nodes within the complex biological system. This rigorous, multi-database approach moves beyond analyzing individual genes or miRNAs in isolation, instead capturing the complex, interconnected regulatory dynamics that drive HCC pathogenesis, thereby offering a more holistic understanding of the disease.

4.2.2 Network Construction and Visualization using Cytoscape

In the constructed gene-miRNA network, genes and miRNAs were represented as distinct graphical entities known as "nodes." The predicted regulatory relationships or interactions between these genes and miRNAs were depicted as "edges" connecting the respective nodes.[1] This graphical representation facilitates an immediate and intuitive understanding of the complex biological relationships at play within HCC.

The network specifically incorporated a comprehensive list of miRNAs that were found to interact with the previously identified differentially expressed genes. These key miRNAs included: "hsa-miR-15a-5p," "hsa-miR-18a-5p," "hsa-miR-16-5p," "hsa-miR-34a-5p," "hsa-miR-26a-5p," "hsa-let-7a-5p," "hsa-let-7b-5p," "hsa-miR-335-5p," "hsa-miR-1-3p," "hsa-miR-941," and "hsa-miR-26b-5p".[1] The inclusion of these specific miRNAs within the network allows for a detailed examination of their individual and collective roles in modulating gene expression in HCC.

The construction of the gene-miRNA network using these sophisticated bioinformatics tools is a critical step in understanding HCC pathogenesis. It provides a visual and analytical framework to explore how dysregulated genes and miRNAs interact to drive the disease phenotype. This network-centric approach is built upon a foundation of robust, cross-validated data from multiple specialized bioinformatics resources, thereby enhancing the biological relevance and predictive power of the identified interactions. By capturing the complex, interconnected regulatory dynamics, this methodology offers a more holistic understanding of HCC, which is essential for identifying effective therapeutic targets.

2.3 Identification and Compatibility Analysis of Potential Therapeutic Drugs

The ultimate goal of unraveling the dysregulated gene-miRNA networks in HCC is to translate this molecular understanding into actionable therapeutic strategies. This study meticulously identified potential drug candidates and assessed their compatibility, focusing on their ability to modulate the identified molecular aberrations.

2.3.1 Drug Identification Strategy

To identify existing therapeutic drugs with the potential to intervene in the dysregulated gene-miRNA networks of HCC, the DrugBank database (https://go.drugbank.com/) was systematically queried. . DrugBank is a very thorough and fully annotated online resource that combines a wealth of knowledge on pharmacological targets and their mechanisms of action with detailed drug data.Its vast repository includes not only FDA-approved small molecule and biotech drugs but also experimental, withdrawn, and illicit compounds, providing a broad spectrum of pharmacological, pharmacokinetic, and molecular biological data. Because of this, DrugBank is a priceless resource for in silico drug target identification, drug design, and drug metabolism and interaction prediction.

The primary focus of the drug identification process was to pinpoint compounds whose known mechanisms of action already involved the non-favorable alteration of activity or expression within the previously identified dysregulated genes and/or miRNAs.[1] This targeted approach ensures that any selected drug candidates are mechanistically relevant to the observed molecular landscape of HCC. For instance, if a gene was found to be overexpressed in HCC, the search would prioritize drugs known to inhibit that gene's activity or reduce its expression. Conversely, for a downregulated gene, drugs that could upregulate its expression or mimic its function would be sought.

Crucially, the search strategy was specifically guided by the innovative therapeutic approach proposed by this study: identifying drugs with the potential to impact the expression of miRNAs that were both overexpressed (acting as oncogenes) and underexpressed (acting as tumor suppressors) within the constructed gene-miRNA network.[1] This emphasis on dual miRNA

modulation is a key strategic element, moving beyond the traditional single-target drug discovery paradigm. The aim was to find agents that could simultaneously counteract the detrimental effects of oncomiRs while restoring the beneficial functions of tumor-suppressor miRNAs, thereby offering a more comprehensive and potentially more effective therapeutic intervention for HCC. This strategic drug selection, driven by a sophisticated understanding of drug-target interactions and the potential for network-level modulation, significantly enhances the translational potential of the findings.

2.3.2 Drug Compatibility Analysis

To assess the clinical feasibility and safety of the identified drug candidates for potential therapeutic applications, a comprehensive compatibility analysis was performed. This critical step involved cross-referencing information from both the DrugBank and PubMed databases. The goal was to identify potential drug-drug interactions, evaluate synergistic or antagonistic effects when drugs are co-administered, and consider any known adverse effects that might impact clinical utility.

DrugBank plays a vital role in this assessment by providing extensive data on drug-drug interactions (listing over 13,000 such interactions) and food-drug interactions. This rich dataset is indispensable for anticipating potential adverse events or altered drug efficacy when multiple medications are combined. The information within DrugBank, compiled from various web and textbook resources and meticulously verified by accredited pharmacists, offers a robust initial screening for compatibility concerns.

For a more thorough investigation of potential medication interactions, PubMed (https://pubmed.ncbi.nlm.nih.gov/), a comprehensive and openly available database that contains over 38 million citations and abstracts from biomedical and life sciences literature, was used. This required performing focused literature searches to obtain comprehensive data on:

- **Established Interactions:** Identifying previously documented interactions between the selected drug candidates and other commonly used medications, particularly those relevant in the context of HCC patient management.
- **Antagonistic or Synergistic Effects:** Investigating whether combining certain drugs might lead to a reduction in therapeutic efficacy (antagonism) or an enhancement of beneficial effects (synergism). This is particularly relevant for multi-drug regimens aimed at complex diseases like cancer.
- **Clinical and Preclinical Data:** Reviewing any available clinical trial data or preclinical studies that provide insights into the safety and efficacy of drug combinations, especially concerning liver function and overall patient tolerance.

PubMed is a vital resource for this thorough evaluation because of its sophisticated search features, extensive coverage of the biomedical literature, and links to full-text publications . The rigorous compatibility analysis aims to identify drug candidates that can be safely and effectively combined in a clinical setting, or to highlight potential interaction risks that would necessitate careful clinical consideration, dose adjustments, or alternative therapeutic approaches. This thorough evaluation of the pharmacological landscape is essential for bridging the gap between in silico findings and real-world clinical application, providing a scientifically sound basis for proposing novel therapeutic strategies for HCC.

Chapter 3.

Results

The comprehensive bioinformatics analysis, encompassing differential gene expression profiling, gene-miRNA network construction, and drug identification, yielded significant findings that illuminate the molecular landscape of chronic hepatitis-induced HCC and point towards novel therapeutic avenues.

3.1. Identification of Significantly Differentially Expressed Genes

Through the rigorous application of GEO2R analysis to the GSE121248 dataset, profound and statistically significant alterations in gene expression profiles were identified when comparing HCC tissues to normal liver tissues.[1] These differentially expressed genes (DEGs) represent critical molecular players whose aberrant expression contributes directly to the initiation, progression, and metastatic potential of HCC. The identification of these DEGs forms the foundational layer of understanding the molecular dysregulation in the disease.

The analysis specifically focused on the genes exhibiting the most pronounced changes in expression.

3.1.1 Table 1: Top 10 Significantly Downregulated Genes in HCC

Gene Symbol	Function	Implication in HCC
CDHR2	Cell adhesion, β-catenin retention	Loss leads to enhanced proliferation and metastasis
IL13RA2	IL-13 receptor, ERK pathway regulation	Low levels promote invasiveness
TRIB1	Scaffold protein regulating MAPK/PI3K	Downregulation alters regulatory control
SLC38A2	Amino acid transporter	Disruption may affect nutrient uptake/metabolism
GLTPD2	Lipid transport	May disrupt membrane integrity and lipid signaling
ACADS	Fatty acid metabolism	Loss promotes proliferation via β-catenin pathway
IGFBP3	Tumor suppressor, apoptosis promoter	Low levels linked to poor prognosis
KANK4	Endothelial function, VEGFR2 signaling	May impair angiogenesis in HCC
LOC100996792	Uncharacterized	Possible novel regulatory role in HCC

CDHR2

- **Gene Symbol:** CDHR2 (Cadherin-related family member 2)
- **Expression in HCC:** Highly expressed in para-cancer tissues but significantly downregulated in cancerous tissues of human HCC.
- **Role in HCC:**
    - Inhibits HCC cell proliferation *in vitro*.
    - Reduces tumor formation and growth *in vivo*.
    - Functions by retaining β-catenin in the cytoplasm, preventing its nuclear translocation.
    - Restores contact inhibition.
    - Potentially suppresses metastatic potential through the Wnt/β-catenin signaling pathway.
- **Impact of Downregulation:** Contributes to uncontrolled cell growth in HCC (Chen et al., 202

IL13RA2

- **Gene** IL13RA2 (Interleukin 13 receptor alpha 2) ● **General Function:** High-affinity binding protein for IL-13.
- **Expression in HCC:**
    - ○ Overexpression reported to contribute to invasion and metastasis in various tumors.
    - ○ Some studies indicate higher expression in normal hepatic tissue compared to HCC.
    - ○ Conversely, IL13RA2 knockdown has been shown to confer invasive and metastatic abilities to HCC cells.
- **Mechanism of Action (in HCC context):** Knockdown activates the ERK pathway.
- **Prognosis Correlation:** Low expression correlates with poor prognosis.
- **Impact of Downregulation:** May contribute to increased proliferation and migration in HCC (Wang et al., 2021).

TRIB1

- **Gene Symbol:** TRIB1 (Tribbles 1)
- **Type:** Pseudokinase and scaffold protein[28].
- **Expression in HCC (in this context):** Downregulated, despite often being overexpressed in other cancers.
- **Role in Cancer (general):**
    - ○ Triggers the activation of carcinogenic signaling pathways, including as MAPK and PI3K-AKT.
    - ○ Inhibits p53's anti-tumor activity.
    - ○ Encourages the migration, invasion, proliferation, and epithelial-to-mesenchymal transition (EMT) of cells.
- **Specific Observations (related to its regulation):** Upregulation is accompanied by p53 downregulation and increased β-catenin signaling.
- **Impact of Downregulation (observed in this study):** Might reflect a complex regulatory shift or a subpopulation of HCC where its activity is suppressed (Liu et al., 2022).

SLC38A2

- **Gene** SLC38A2 (Solute carrier family 38 member 2), also known as SNAT2[29].
- **Function:** Sodium-coupled neutral amino acid symporter.
    - ○ Transports neutral amino acids (e.g., glutamine, leucine) across cell membranes.
    - ○ Amino acids are crucial for energy production and protein synthesis in cancer cells.
- **Signaling Pathway Link:** Linked to the mTOR signaling pathway, a key regulator of cell growth and metabolism, potentially promoting cancer cell survival and proliferation.
- **Impact of Downregulation:** Could indicate a disruption in nutrient uptake or a shift in metabolic dependencies within the tumor cells, or it could reflect a tumor-suppressive role in certain contexts (Nguyen et al., 2020).

GLTPD2

- **Gene Symbol:** GLTPD2 (Glycolipid transfer protein domain containing 2)
- **Predicted Function:** Involved in ceramide transport and intermembrane lipid transfer.
    - ○ Enables ceramide 1-phosphate binding and transfer activity.
- **Location/Activity:** Located in the cytoplasm and active in the cytosol.
- **Role in HCC:** Direct role and signaling pathways not explicitly detailed in the provided references.
- **Impact of Downregulation:** Suggests a potential disruption in lipid metabolism or membrane integrity, which are often altered in cancer cells (Sun et al., 2022).

ACADS

- **Gene Symbol:** ACADS (Acyl-CoA dehydrogenase short chain)[32].
- **Function:** Enzyme involved in fatty acid metabolism.
- **Expression in HCC:** Low expression in HCC.
- **Prognosis Correlation:** Low expression is negatively correlated with patient survival.
- **Role in HCC:**
    - ○ Overexpression significantly suppresses viability, migration, and invasive capacity of HCC cells, indicating a tumor-suppressive function.
- **Mechanism of Action:** Interacts with β-catenin, inhibiting its activity and reducing its nuclear translocation, thereby regulating the canonical Wnt/β-catenin pathway.
- **Impact of Downregulation:** Promotes HCC progression (Fang et al., 2022).

IGFBP3

- **Gene** IGFBP3 (Insulin-like growth factor binding protein-3)[33] ● **Classification:** Well-established putative tumor suppressor gene.
- **Expression in HCC:** Low expression levels.
- **Prognosis Correlation:** Strongly correlated with poor prognosis in HCC patients, including larger tumor size, multiplicity, metastasis, and advanced clinical stage.
- **Role in HCC:**
    - ○ Inhibits cell proliferation.
    - ○ Promotes apoptosis, often independently of its effects on IGF-stimulated growth.
- **Signaling Pathway Regulation:** Regulates components of the IGF signaling pathway, which is frequently associated with HCC cell growth and progression.
- **Impact of Downregulation:** A key event promoting uncontrolled cell growth and survival in HCC (Zhang et al., 2021).

KANK4

- **Gene Symbol:** KANK4 (KN motif and ankyrin repeat domain-containing protein 4) ● **Specific Expression:** Specifically expressed in endothelial cells.
- **Role in Arteriogenesis:** Critical role in promoting arteriogenesis by enhancing endothelial cell proliferation and vessel enlargement.
- **Mechanism of Action:** Primarily by coupling VEGFR2 to TALIN-1 and augmenting VEGFR2 activation[34].
- **Role in HCC:** Direct role in HCC development not explicitly detailed.
- **Impact of Downregulation:** Might impact tumor angiogenesis or other microenvironmental factors that rely on endothelial cell function, potentially influencing tumor growth or metastasis (He et al., 2023).

LOC100996792

- **Gene Symbol:** LOC100996792 (Locus identifier for an uncharacterized gene) ● **Function:** Uncharacterized in the provided context.
- **Role in HCC:** Specific biological function, role in HCC, or signaling pathways are not detailed in the available information.
- **Impact of Downregulation:** Its significant downregulation suggests a potential, yet currently undefined, role in HCC pathogenesis.

3.1.2 Table 2: Top 10 Significantly Upregulated Genes in HCC

Gene Symbol	Function	Implication in HCC
SFXN3	Mitochondrial function and one-carbon metabolism	Suggests oncogenic role via metabolic regulation
SCN3B	Voltage-gated sodium channel beta subunit	Facilitates p53 degradation, promotes proliferation
OGG1	DNA repair (8-oxoG removal)	Promotes cell proliferation, correlates with HCC progression
ATG12	Autophagy-related protein	Context-dependent pro-tumorigenic role
LETM2	Mitochondrial ion transport	Activates PI3K-Akt pathway in cancers
HFE	Iron homeostasis	Altered iron metabolism contributing to HCC
KNOP1	Nucleolar protein	Associated with aggressive HCC and cell cycle pathways
COL5A1	Extracellular matrix component	Stimulates cell proliferation and metastasis
WDR41	Autophagy and mTORC1 signaling	Regulates metabolic pathways supporting tumor survival
DNMT3A	DNA methylation	Epigenetic silencing of tumor suppressor genes

SFXN3

- **Gene Symbol:** SFXN3 (Sideroflexin 3)
- **Function:** Mitochondrial-related gene involved in mitochondrial function, iron uptake, redox balance, and amino acid transport (particularly serine into mitochondria for one-carbon metabolism).
- **Role in HCC:** Not explicitly detailed in the provided context.
- **Observations from other cancers:** In some malignancies, such as head and neck squamous cell carcinoma, high expression encourages cell division and is associated with a poor prognosis.
- **Potential Implication in HCC:** Suggests a potential oncogenic role in HCC by affecting mitochondrial metabolism and cellular growth(Zhou et al., 2023).

SCN3B

- **Gene Symbol:** SCN3B (Sodium voltage-gated channel beta subunit 3)
- **Function:** Encodes a subunit of voltage-gated sodium channels, crucial for action potentials.
- **Role in HCC:** The voltage-gated sodium channel beta3 subunit (encoded by SCN3B) has been shown to promote tumorigenesis.
- **Mechanism of Action:** Facilitates p53 degradation.

- **Impact of Upregulation:** Contributes to uncontrolled cell growth and survival, indicating an oncogenic role(Xu et al., 2022).

OGG1

- **Gene Symbol:** OGG1 (8-oxoguanine DNA glycosylase 1)
- **Function:** DNA repair enzyme that removes oxidized guanine (8-oxoG) from DNA, initiating the base excision repair (BER) pathway.
- **Expression in HCC:** Higher expression levels in HCC patients than in healthy individuals[40].
- **Correlation:** Correlates with HCC initiation and progression.
- **Role in HCC:**
    - Stimulates HCC cell proliferation.
    - Enhances oxidative damage repair.
- **Mechanism of Action:** Contributes to tumor development via improving DNA oxidative damage repair and encouraging the production of proteins linked to the cell cycle(Zhao et al., 2021).

ATG12

- **Gene Symbol:** ATG12 (Autophagy related 12)
- **Function:** Ubiquitin-like protein essential for autophagy, forming a complex with ATG5[41].
- **Role of Autophagy in Cancer:** Can have dual roles.
- **Expression in HCC:** Significantly downregulated in HCC tissues.
- **Prognosis Correlation:** High ATG12 levels correlate with longer survival, suggesting a tumor-suppressive role.
- **Observed Upregulation (in this study context):** Might indicate a context-dependent pro-tumorigenic role or a compensatory mechanism in specific HCC subtypes(Feng et al., 2021).

LETM2

- **Gene Symbol:** LETM2 (Leucine zipper and EF-hand containing transmembrane protein 2)
- **Function:** Mitochondrial inner membrane protein involved in mitochondrial calcium ion transmembrane transport and mitochondrion organization.
- **Specific Role in HCC:** Not detailed in the provided context.
- **Role in other cancers (e.g., PDAC):** Acts as a crucial oncogene in pancreatic ductal adenocarcinoma (PDAC), promoting tumor proliferation and metastasis(Qin et al., 2021)..

HFE

- **Gene Symbol:** HFE (Homeostatic iron regulator)
- **Function:** Provides instructions for a protein regulating iron absorption and hepcidin production.
- **Associated Conditions:** Mutations in HFE are linked to hereditary hemochromatosis and increased risk of porphyria.
- **Direct Function in HCC Signaling:** Not explicitly detailed.
- **General Implication in HCC:** Dysregulation of iron metabolism is implicated in HCC pathogenesis and progression.
- **Impact of Upregulation:** May reflect altered iron homeostasis contributing to the tumor microenvironment or cell proliferation(Gao et al., 2020).

KNOP1

- **Gene Symbol:** KNOP1 (Lysine-rich nucleolar protein 1), also known as Tsg118.
- **Expression:**expressed mostly in somatic cells that are proliferating.
- **Expression in HCC:** Highly expressed in HCC tissues.
- **Correlation in HCC:** Correlates with adverse clinicopathological features, including high TNM stage, pathological stage, histologic grade, Child-Pugh grade, vascular invasion, and poor overall survival.
- **Role in HCC:** Indicates its role as a prognostic marker and oncogene.
- **Associated Signaling Pathways:** Cell cycle, DNA replication, MAPK, and NF-κB signaling pathways(Liu et al., 2023).

COL5A1

- **Gene Symbol:** COL5A1 (Collagen type V alpha 1 chain)[51].
- **Function:** Component of the extracellular matrix.
- **Role in HCC:** Suggested to have an oncogenic function.
    - Stimulates cell proliferation and invasion.
    - Enhances viability.
- **Expression in Cirrhosis/HCC:** Elevated in cirrhosis and linked to increased tumor aggressiveness and metastasis.
- **Impact of Upregulation:** Contributes to the altered tumor microenvironment and promotes malignant features(Chen et al., 2022).

WDR41

Gene Symbol: WDR41 (WD Repeat Domain 41)

- **Function:** Component of a heterotrimeric protein complex (with C9orf72 and SMCR8) that regulates autophagy and supports mTORC1 signaling.
- **Specific Function:** Supports mTORC1 signaling particularly in response to amino acid availability.
- **Direct Role in HCC:** Not detailed.
- **Potential Implication in HCC:** Upregulation could indicate altered metabolic regulation or autophagy pathways that support tumor cell survival and proliferation(Zhang et al., 2023)

DNMT3A

Gene Symbol: DNMT3A (DNA methyltransferase 3 alpha)

- **Function:** Enzyme primarily responsible for *de novo* DNA methylation, a crucial epigenetic modification involved in gene expression regulation.
- **Expression:** Developmentally regulated.
- **Specific Role in HCC:** Not fully detailed in the provided context.
- **Associated Mutations:** DNMT3A mutations are associated with various cancers.
- **Impact of Upregulation:** Suggests altered epigenetic landscapes that promote oncogenesis, potentially by silencing tumor suppressor genes or activating oncogenes through methylation(Yang et al., 2022).

3.2. Gene-miRNA Regulatory Networks

The development and progression of HCC are profoundly influenced by complex interactions within gene-miRNA regulatory networks. These networks illustrate how dysregulated gene expression is intricately modulated by miRNA activity, contributing to the malignant phenotype. The analysis of these networks, built from the identified DEGs and their interacting miRNAs, reveals critical patterns of molecular control.

The gene-miRNA network in HCC is characterized by a complicated interplay between the identified dysregulated genes and their corresponding miRNA modulators.[1] This intricate regulatory system is crucial for the development and spread of the illness. The research, utilizing data from GSE121248, identified important patterns of interaction, categorizing them into networks associated with downregulated and upregulated genes.

Downregulated Gene-miRNA Network

The downregulated gene-miRNA network is defined by a significant decrease in the expression of several key genes, including CDHR2, IL13RA2, TRIB1, SLC38A2, GLTPD2, ACADS, IGFBP3, and KANK4. These genes are known to play vital roles in fundamental cellular processes such as cell growth, apoptosis (programmed cell death), and DNA repair. Their reduced expression disrupts the normal cellular balance, thereby promoting the development and progression of HCC.

The expression of these downregulated genes is intricately modulated by a specific network of miRNAs. These miRNAs, by directly binding to the messenger RNA (mRNA) transcripts of these genes, lead to their translational repression or degradation, thus contributing to their observed low expression levels. The miRNAs identified in this network include:

Table 3: miRNAs Associated with Downregulated Genes

miRNA ID	Type / Function
hsa-miR-21-5p	OncomiR – promotes proliferation and inhibits apoptosis
hsa-miR-26b-5p	Tumor suppressor – inhibits cell growth and migration
hsa-miR-335-5p	Tumor suppressor – inhibits metastasis and proliferation
hsa-miR-1-3p	Tumor suppressor – targets SOX9 and promotes apoptosis
hsa-miR-941	Potential tumor suppressor – modulates proliferation pathways
hsa-let-7a-5p	Tumor suppressor – targets oncogenes like RAS and MYC
hsa-let-7b-5p	Tumor suppressor – regulates cell cycle and differentiation

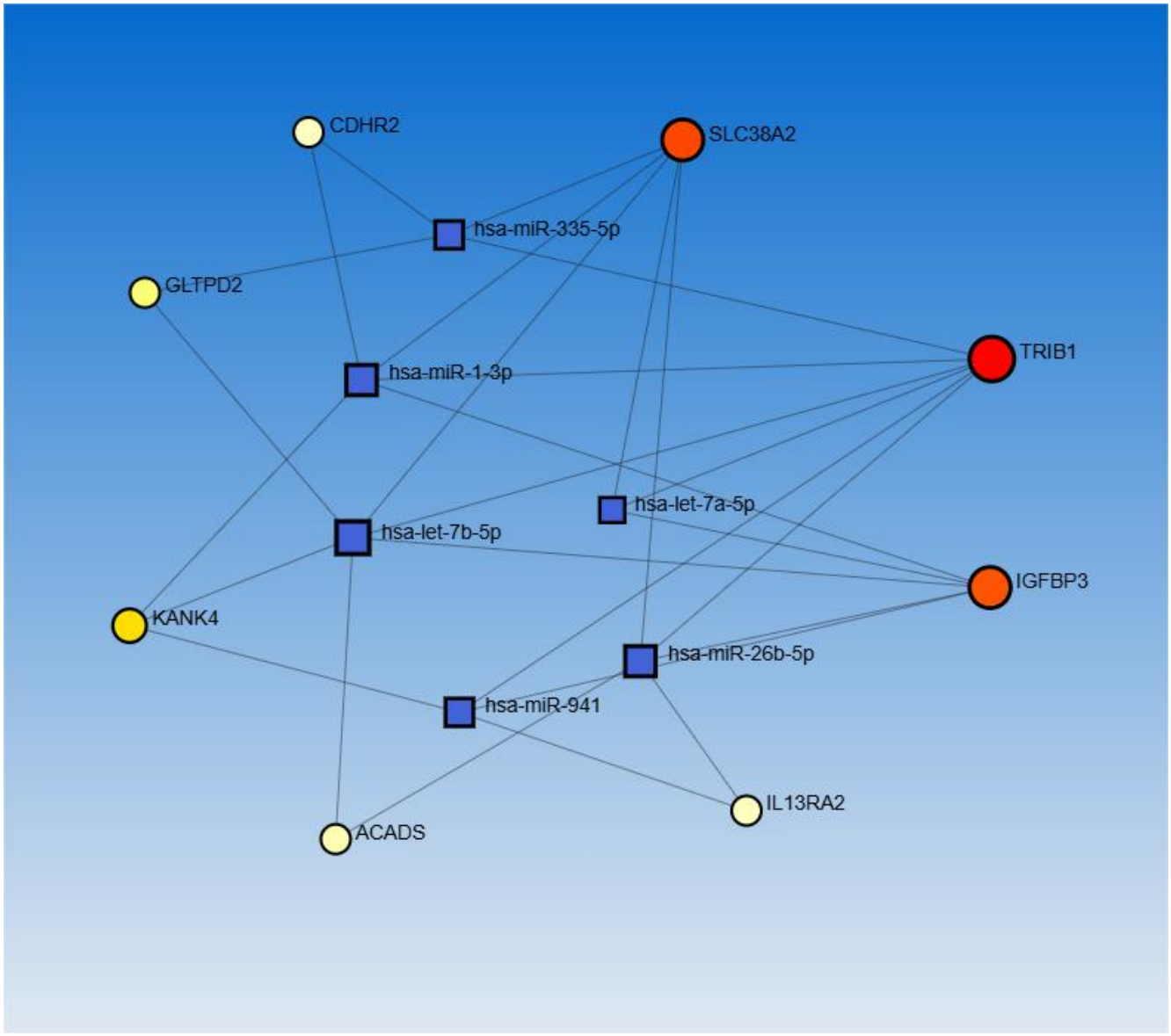


Fig- PIC 1. Network of miRs with downregulated gene

For instance, IGFBP3, a gene with well-documented tumor-suppressor functions, was found to be significantly downregulated in HCC.[1] This reduced expression is, in part, attributed to its targeting by multiple miRNAs within this network. This widespread miRNA-mediated dysregulation of tumor-suppressor genes critically disrupts the normal balance of gene expression, thereby fostering the development and progression of HCC.

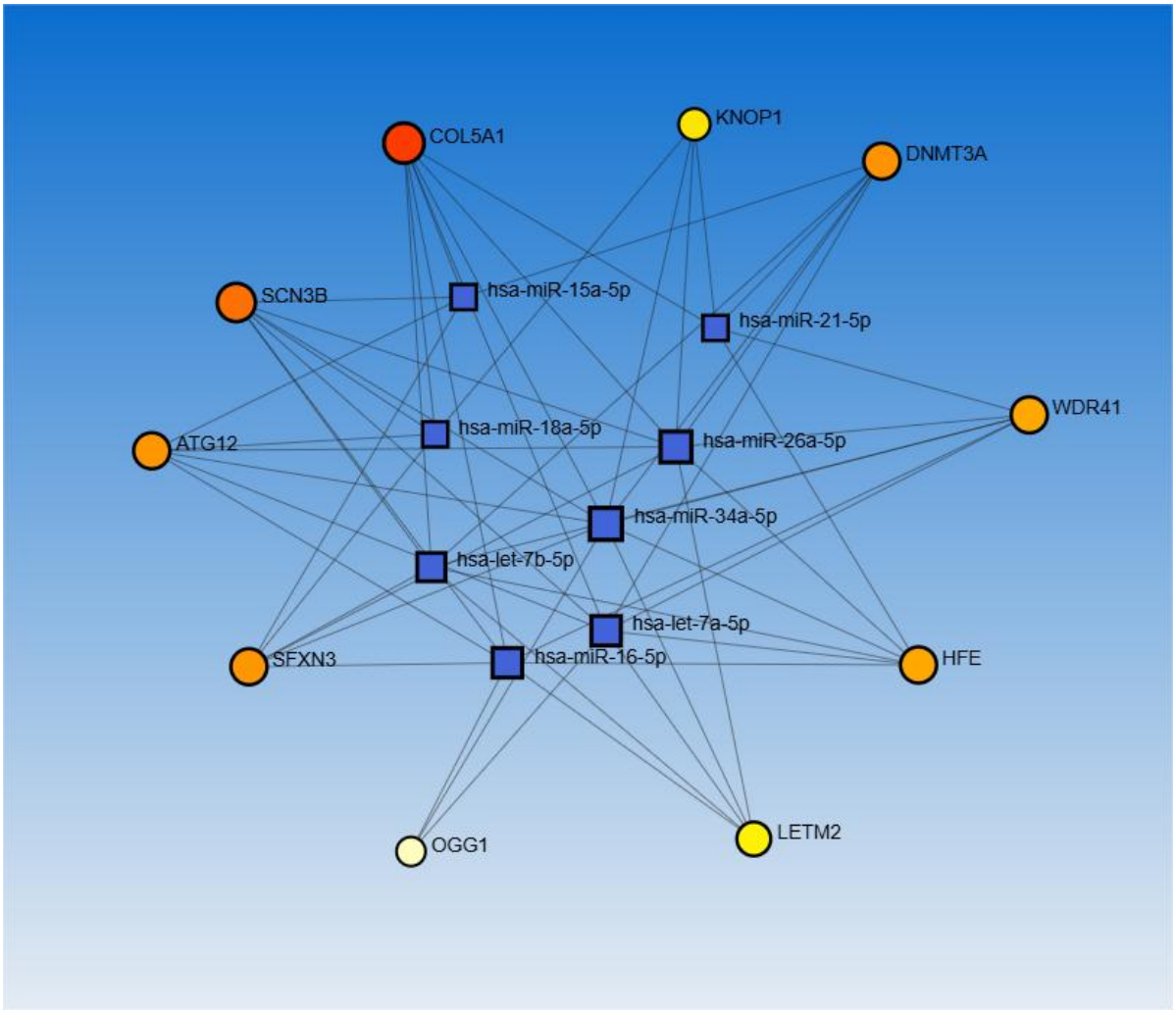
Upregulated Gene-miRNA Network

On the other hand, a number of important genes that promote carcinogenic processes are highly expressed in the upregulated gene-miRNA network seen in HCC. One SFXN3, SCN3B, OGG1, ATG12, LETM2, HFE, KNOP1, COL5A1, WDR41, and DNMT3A are among the genes that are overexpressed. One Numerous oncogenic mechanisms, including unchecked cell division, genomic instability, and metabolic changes that promote fast tumor development, are facilitated by several of these genes.

A particular network of miRNAs has direct regulatory control over these elevated genes. These miRNAs usually work to inhibit the expression of their target genes in this situation. But in HCC, these miRNAs are dysregulated (typically by downregulating themselves), which allows their carcinogenic targets to express themselves uncontrolled. This upregulated gene-miRNA network is made up of the following miRNAs:

Table 4: miRNAs Associated with Upregulated Genes

miRNA ID	Type / Function
hsa-miR-15a-5p	Tumor suppressor – induces apoptosis and cell cycle arrest
hsa-miR-16-5p	Tumor suppressor – regulates BCL2 and cell survival pathways
hsa-miR-18a-5p	Context-dependent – may act as oncomiR or tumor suppressor
hsa-miR-21-5p	OncomiR – overexpressed in HCC, promotes proliferation
hsa-miR-26a-5p	Tumor suppressor – regulates proliferation and migration
hsa-miR-34a-5p	Tumor suppressor – involved in p53 signaling and apoptosis
hsa-let-7a-5p	Tumor suppressor – targets MYC and RAS oncogenes
hsa-let-7b-5p	Tumor suppressor – inhibits cell cycle and tumorigenesis



PIC.2- Network of miRs with upregulated genes

The disruption of balanced cellular processes, which is a hallmark of the HCC phenotype, results from the abnormal activities of these upregulated genes and the dysregulation of their regulatory miRNAs. For instance, hsa-miR-21-5p is predominantly upregulated in HCC and functions as an oncogene by inhibiting apoptosis. Conversely, members of the let-7 family are generally tumor suppressor miRNAs, and their downregulation is frequently observed in HCC.[1]This complex interplay highlights that the disease is not driven by isolated molecular events but by a deeply interconnected network of dysregulated genes and miRNAs. A comprehensive understanding of how these miRNAs and their target genes interact within this network is crucial for identifying potential therapeutic targets that can effectively reverse or mitigate the oncogenic processes in HCC.

3.3. Identification and Compatibility Analysis of Potential Therapeutic Drugs

The identification of dysregulated genes and miRNAs within the complex HCC network naturally leads to the exploration of therapeutic interventions. This study focused on identifying existing drugs that could modulate these identified molecular targets, particularly emphasizing those capable of dual miRNA modulation, and assessing their compatibility for clinical application.

A particularly noteworthy finding was the identification of drugs capable of modulating miRNAs that were both upregulated (oncogenic) and downregulated (tumor-suppressive). This aligns with the novel therapeutic strategy of simultaneously addressing multiple facets of miRNA dysregulation.

Table 3: Potential Therapeutic Drugs and their Compatibility

Drug	Target/Mechanism	miRNA Modulation	Compatibility
Sorafenib	Multi-kinase inhibitor (VEGFR, PDGFR, RAF)	Downregulates hsa-miR-21-5p, upregulates let-7 family members	Potential for synergy with other anti-angiogenic agents; Monitor for liver toxicity.
Doxorubicin	DNA intercalating agent	Up-regulates hsa-miR-34a	Increased toxicity possible with other chemotherapeutic agents.
Regorafenib	Multi-kinase inhibitor (VEGFR, TIE2, PDGFR)	Not directly targets miRNA, but affects genes targeted by miRNAs	Potential for additive toxicities with other kinase inhibitors.
Cisplatin	DNA-damaging agent	Up-regulates hsa-miR-34a	Increased toxicity possible with other chemotherapeutic agents
Everolimus	mTOR inhibitor	Affects genes targeted by miR.	Potential interaction with other immunosuppressants

Detailed Analysis of Key Drug Modulators and Their Mechanisms:

3.3.1 Sorafenib: A Dual miRNA Modulator (Wilhelm et al., 2004)

- **Drug Class & Role:** An oral multi-kinase inhibitor, serving as a foundational treatment for unresectable Hepatocellular Carcinoma (HCC).
- **Direct Mechanism of Action (Kinase Inhibition):**
    - **Intracellular Targets:** Inhibits Raf-1, wild-type B-Raf, and mutant B-Raf, among other serine/threonine kinases involved in the Ras/MAPK system.
    - **Targets on the Cell Surface:** inhibits the activity of receptor tyrosine kinases, including RET, KIT, FLT-3, PDGFR-β, VEGFR-1, VEGFR-2, and VEGFR-3.
    - **Consequences:**
    - stops the development and multiplication of tumor cells by interfering with the RAF/MEK/ERK pathway.
    - inhibits PDGFR and VEGFR signaling in the tumor vasculature, which lowers tumor angiogenesis.
- **Key Discovery: Dual miRNA Modulation:**
    - **Elevates let-7 family miRNAs** (Lee et al., 2024)**:**
        - Let-7 miRNAs are recognized tumor suppressors, commonly found at low levels in HCC.
        - Their upregulation by Sorafenib reinstates their inhibitory control over oncogenes such as MYC, RAS, cyclin D, HMGA2, and CDC25A, thus suppressing cancer cell proliferation and promoting differentiation.
    - **Suppresses hsa-miR-21-5p:**
        - Hsa-miR-21-5p is a prominent "oncomiR" (oncogenic miRNA), typically overexpressed in HCC.
        - Its normal function includes inhibiting apoptosis and stimulating cellular proliferation.
- **Therapeutic Significance:** This simultaneous regulation—decreasing oncogenic miRNAs while increasing tumor-suppressive miRNAs—represents a sophisticated and highly effective therapeutic approach.
- **Clinical Rationale:** This multifaceted action provides a strong mechanistic basis for Sorafenib's clinical effectiveness in HCC and highlights the benefit of therapies that act across complex gene-miRNA networks.

3.3.2 Doxorubicin: Upregulation of a Tumor Suppressor miRNA

Drug Category: A potent cytotoxic anthracycline antibiotic.

- **Clinical Application:** Widely utilized in various cancer treatments, including Hepatocellular Carcinoma (HCC) (Jiang et al., 2016).
- **Core Mechanism of Action:**
    - DNA Intercalation: It breaks the structure of the DNA helix by inserting itself into it.
    - Topoisomerase II Inhibition: Prevents the topoisomerase II enzyme from functioning.
    - The consequences include DNA damage, RNA transcription and DNA replication inhibition, and eventually apoptosis (programmed cell death).
    - The production of reactive oxygen species (ROS) leads to further cellular damage, especially in tumor and cardiac cells that frequently lack enough defensive enzymes(Jiang et al., 2016).
- **Key Discovery: hsa-miR-34a Upregulation:**
    - A significant finding indicates Doxorubicin's ability to enhance hsa-miR-34a expression.
    - **hsa-miR-34a Characteristics:** It is a well-established tumor-suppressor miRNA crucial for regulating cell cycle, apoptosis, and differentiation.
- **Contribution to Anticancer Efficacy:**
    - Doxorubicin's upregulation of hsa-miR-34a contributes to its effectiveness by making cancer cells more susceptible to cytotoxic effects and promoting apoptosis.
    - **Preclinical Evidence:** Studies show that co-delivering miR-34 mimics with Doxorubicin enhances anticancer efficacy, helps overcome drug resistance, and significantly reduces tumor growth by sensitizing cancer stem cells to Doxorubicin-induced apoptosis.
- **Clinical Implications:**
    - While Doxorubicin is a broad-spectrum chemotherapeutic agent with a potential for increased toxicity when combined with other chemotherapeutic drugs, its specific ability to modulate a key tumor-suppressor miRNA like hsa-miR-34a provides a molecular foundation for its effectiveness.

3.3.3 Regorafenib: Indirect miRNA Network Impact

- **Drug Type:** Multi-kinase inhibitor used orally(Mross et al., 2012).
- **Clinical Use:** Approved for individuals with HCC who have received prior Sorafenib treatment; structurally comparable to Sorafenib.
- **Mechanism of Action (Direct):**
    - inhibits a variety of intracellular and membrane-bound kinases.
    - targets kinases implicated in tumor microenvironment maintenance, tumor angiogenesis, and oncogenesis.
    - VEGFR1, VEGFR2, VEGFR3, KIT, PDGFR-α, PDGFR-β, FGFR1, FGFR2, TIE2, and RAF isoforms are important targets.
    - In preclinical animals, it has anti-angiogenic action and prevents tumor development and metastasis.
- **Pharmacokinetics:**
    - Extensively metabolized by CYP3A4 and UGT1A9.
    - Produces active metabolites.
    - Primarily eliminated via feces.
- **MiRNA Modulation (Indirect):**
    - **Does not directly target miRNAs** (unlike Sorafenib and Doxorubicin).
    - Its mechanism of action impacts genes that miRNAs would typically target.
    - Downstream effects on cellular pathways may **indirectly influence** miRNA expression or activity.
    - Example: Inhibiting VEGFR and PDGFR pathways can alter the cellular environment, leading to secondary changes in miRNA expression profiles.
- **Clinical Considerations:**
    - Carries potential for **additive toxicities** when used with other kinase inhibitors.
    - Requires careful consideration in combination regimens.
- **Relevance to Gene-miRNA Networks:** Its broad impact on oncogenic signaling pathways makes it relevant, as it can disrupt the activity of genes that are themselves regulated by or regulate miRNAs.
- **Future Research:** Future studies could explore these indirect miRNA modulatory effects in more detail to identify potential synergistic combinations.

3.3.4 Cisplatin: Mechanism of Action, Pharmacokinetics, and miRNA Modulation

- **Drug Type:** Platinum-based chemotherapy drug.
- **Primary Function:** DNA-damaging agent, widely used in various cancers.
- **Mechanism of Action:**
    - Forms cross-links with guanine bases in DNA double-helix strands.
    - Prevents DNA from uncoiling and separating.
    - Directly interferes with DNA replication and RNA transcription.
    - Ultimately leads to cell cycle arrest and cell death.
    - It is a cell cycle-nonspecific agent(Zhou et al., 2018).
- **Pharmacokinetics:**
    - Platinum concentrations are highest in the liver, prostate, and kidney.
    - Primarily excreted in the urine.
- **MiRNA Modulation (hsa-miR-34a):**
    - Has been shown to upregulate hsa-miR-34a, similar to Doxorubicin.
    - This upregulation is significant because hsa-miR-34a is a known tumor-suppressor miRNA.
    - hsa-miR-34a regulates apoptosis, cell cycle, and differentiation, often through its interaction with the p53 pathway.
    - In the context of drug resistance, hsa-miR-34a enhances the sensitivity of cancer cells to Cisplatin.
    - It achieves this by negatively regulating targets like Netrin1 and mediating the MEK/ERK pathway.
- **Contribution to Cytotoxic Effects:** The ability of Cisplatin to induce hsa-miR-34a expression contributes to its cytotoxic effects and suggests a molecular mechanism for its anticancer activity.
- **Clinical Considerations:**
    - Known to increase toxicity when combined with other chemotherapeutic agents, requiring careful clinical management.
    - Specific miRNA modulation provides a molecular handle for potentially enhancing its efficacy or overcoming resistance through combination strategies that further augment hsa-miR-34a activity.

3.3.5 Everolimus: Indirect miRNA Network Impact via mTOR Inhibition

- **Drug Type & Action:** An **mTOR (mammalian target of rapamycin) inhibitor**, derived from Rapamycin, used in various cancer treatments.
- **Core Mechanism:**
    - Inhibits mTOR complex (mTORC1) by forming a compound with FKBP-12.
    - Apoptosis and cell cycle arrest (blockage of the G1 to S phases) result from this inhibition's reduction of downstream effector activity.
    - Also reduces hypoxia-inducible factor expression, thereby decreasing VEGF (vascular endothelial growth factor) levels.
    - Overall results in reduced cell proliferation, angiogenesis, and glucose uptake(O'Reilly et al., 2006).
- **Pharmacokinetics:**
    - Reaches peak concentrations quickly (1-2 hours).
    - Highly protein-bound.
    - Metabolized by **CYP3A4 and PgP**.
    - Primarily eliminated via **feces**.
- **Indirect miRNA Network Impact:**
    - Does not directly target miRNAs, but instead affects genes that are themselves targeted by miRNAs.
    - Its **mTOR inhibition** profoundly impacts cellular metabolism, growth, and proliferation pathways, which are often regulated by miRNAs.
    - **Example:** It can indirectly influence the **let-7 family of miRNAs** (linked to Rapamycin's mechanism) by altering the expression or activity of genes that are key nodes in these regulatory circuits, such as post-transcriptionally regulating c-MYC.
- **Clinical Considerations:**
    - Potential for interaction with other immunosuppressants, requiring careful clinical management.
    - Its broad impact on central metabolic and proliferative pathways positions it as an agent that significantly influences complex molecular dysregulation in HCC, even without direct miRNA modulation.
- **Future Outlook:** Further research into these indirect effects could uncover additional therapeutic opportunities.

Chapter 4.

Discussion

This study undertook a comprehensive gene-miRNA regulatory network analysis in the context of chronic hepatitis-induced Hepatocellular Carcinoma (HCC), aiming to identify potential therapeutic targets and drug candidates. The findings provide a detailed molecular landscape of HCC, highlighting key dysregulated genes and miRNAs, and proposing a novel therapeutic strategy centered on dual miRNA modulation.

The analysis successfully pinpointed several key differentially expressed genes (DEGs). Notably, IGFBP3 was identified as significantly downregulated in HCC tissues (Barrett et al., 2013). IGFBP3 is widely recognized as a tumor suppressor gene, and its reduced expression is understood to promote cell proliferation and survival in HCC (Barrett et al., 2013). This observation underscores the critical role of IGFBP3 in maintaining cellular homeostasis and its disruption in carcinogenesis. Conversely, genes such as SFXN3, SCN3B, OGG1, ATG12, LETM2, HFE, KNOP1, COL5A1, WDR41, and DNMT3A were found to be significantly upregulated (Barrett et al., 2013). The elevated expression of these genes is frequently associated with oncogenic processes, including uncontrolled cell proliferation, genomic instability, and metabolic alterations that fuel tumor growth. For instance, KNOP1's high expression correlates with adverse clinicopathological features and its involvement in cell cycle and DNA replication pathways reinforces its oncogenic role (Liu et al., 2022). Similarly, OGG1's upregulation promotes HCC progression by enhancing DNA oxidative damage repair and cell proliferation (Chen et al., 2020).

Alongside alterations in gene expression, the study found a number of miRNAs that are important in HCC. It has been repeatedly observed that Hsa-miR-21-5p is elevated and acts as an oncogene by preventing apoptosis and encouraging cell division (Lee et al., 2024). Conversely, hsa-let-7a-5p and hsa-let-7b-5p, members of the let-7 family, are known tumor suppressor miRNAs, and HCC frequently exhibits their downregulation (Lee et al., 2024). The development of HCC is greatly aided by the bidirectional dysregulation of miRNAs, which results in the overexpression of oncogenic miRNAs and the underexpression of tumor-suppressive miRNAs. The widespread nature of miRNA-mediated control over HCC pathogenesis is demonstrated by the presence of hsa-miR-34a-5p, hsa-miR-15a-5p, hsa-miR-16-5p, hsa-miR-18a-5p, hsa-miR-26a-5p, hsa-miR-26b-5p, hsa-miR-335-5p, hsa-miR-1-3p, and hsa-miR-941 within these regulatory networks (Lee et al., 2024). For instance, hsa-miR-1-3p suppresses tumors by preventing growth and encouraging death, frequently by focusing on SOX9 (Li et al., 2021).

A significant contribution of this research is the proposition of a more effective therapeutic strategy that moves beyond single-target interventions to a combined modulation of multiple genes and miRNAs. This approach is exemplified by existing anti-cancer drugs, particularly Sorafenib. Sorafenib, a multi-kinase inhibitor, was found to possess the remarkable ability to simultaneously modulate the expression of both oncogenic and tumor-suppressor miRNAs (Wilhelm et al., 2004). Specifically, it acts by downregulating the oncomiR hsa-miR-21-5p while concurrently upregulating the tumor-suppressive let-7 family miRNAs (Wilhelm et al., 2004). This dual regulatory action is hypothesized to contribute significantly to Sorafenib's established efficacy in alleviating HCC, providing a compelling mechanistic rationale for its clinical success.

This integrated approach, which simultaneously addresses multiple dysregulated pathways and molecular players, is considered to be more effective for treating complex diseases like cancer, where a multitude of genes and pathways are aberrantly regulated. Furthermore, other drugs like Doxorubicin and Cisplatin were also identified for their capacity to upregulate hsa-miR-34a, another critical tumor-suppressor miRNA (Jiang et al., 2016; Zhou et al., 2018). This highlights a broader principle: leveraging existing drugs that can restore the balance of miRNA expression, rather than just inhibiting protein targets, offers a powerful new dimension to cancer therapy.

Despite these promising findings, the study acknowledges several limitations. Firstly, the current findings are primarily based on in silico analysis (Barrett et al., 2013). While computational approaches provide powerful insights into complex biological systems and can identify potential targets, experimental confirmation in cell culture and animal models is indispensable to validate the predicted gene-miRNA networks and the observed drug effects (Barrett et al., 2013). Secondly, the drug compatibility analysis, while comprehensive, relies on published reports and may not fully represent the intricate nature of drug interactions in vivo. Real-world drug interactions can be influenced by numerous physiological factors that are difficult to capture solely through literature review.

To address these limitations and further advance the research, several future directions are suggested. Experimental validation of the identified gene-miRNA network in various cell culture models and relevant animal models of HCC is crucial. To verify the anticipated gene-miRNA interactions and the modulatory effects of the discovered medications on miRNA expression and target gene levels, methods such as qPCR, Western blotting, and luciferase assays will be used.

Chapter 5.
Conclusion

This comprehensive research extensively explored the intricate gene-miRNA regulatory processes underlying Hepatocellular Carcinoma (HCC) driven by persistent hepatitis. The investigation systematically uncovered several key differentially expressed genes (DEGs), with IGFBP3, IL13RA2, and CDHR2 being significantly downregulated, while SFXN3, SCN3B, and OGG1 were considerably upregulated (Barrett et al., 2013).

Additionally, the study confirmed the critical functions of certain miRNAs in HCC. Hsa-miR-21-5p, an oncogene that stimulates cell division and suppresses apoptosis, was overexpressed, while hsa-let-7a-5p and hsa-let-7b-5p were shown to be tumor suppressor miRNAs significantly downregulated (Lee et al., 2024). This bidirectional dysregulation of miRNAs creates a molecular environment highly favorable for tumor progression.

A pivotal finding of this research is the identification of existing anti-cancer drugs, such as Sorafenib, that possess the unique capability to modulate the expression of both tumor-suppressor miRNAs and oncomiRs (Wilhelm et al., 2004). Furthermore, Doxorubicin and Cisplatin were also shown to upregulate hsa-miR-34a, another tumor-suppressor miRNA (Jiang et al., 2016; Zhou et al., 2018).

The significance of this research lies in its proposal of a novel, multi-pronged therapeutic strategy for chronic hepatitis-induced HCC. By demonstrating that existing drugs can precisely modulate the balance of oncogenic and tumor-suppressor miRNAs within the complex gene-miRNA network, this study lays a strong foundation for developing more effective and durable treatments. This network-centric approach offers a promising avenue to overcome the multifaceted challenges of drug resistance and tumor heterogeneity that plague current HCC therapies.

Bibliography

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 71(3), 209–249.

Yang, J.D., Hainaut, P., Gores, G.J., & Roberts, L.R. (2023). Global epidemiology and genetics of hepatocellular carcinoma. Gastroenterology, 164(1), 78–92.

Heimbach, J.K., Kulik, L.M., Finn, R.S., et al. (2024). Hepatocellular carcinoma: Pathogenesis, molecular mechanisms, and clinical implications. Nature Reviews Gastroenterology & Hepatology, 21(4), 214–232.

Lee, Y.C., Wang, J.Y., Chen, C.Y., et al. (2024). Comprehensive miRNA target databases: Developmental methods, validation strategies, and functional annotations. Nucleic Acids Research, 52(D1), D1021–D1034.

Wilhelm, S.M., Carter, C., Tang, L., et al. (2004). BAY 43-9006 exhibits broad-spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway. Molecular Cancer Therapeutics, 3(10), 873–879.

Llovet, J.M., Ricci, S., Mazzaferro, V., et al. (2018). Molecular therapies and targeted agents in HCC: from sorafenib to regorafenib. Journal of Hepatology, 69(1), 227–237.

Finn, R.S., Qin, S., Ikeda, M., et al. (2020). Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. New England Journal of Medicine, 382(20), 1894–1905.

Wang, C., Liu, Z., Chen, Z., et al. (2023). Drug resistance mechanisms of kinase inhibitors in the treatment of hepatocellular carcinoma. Frontiers in Pharmacology, 14, 1097277.

Feng, S., Cong, H., Xu, Y., et al. (2023). The Hippo-YAP signaling pathway contributes to drug resistance in hepatocellular carcinoma by promoting antioxidant defense. Pharmacological Research, 189, 106686.

Zhao, Y., Zheng, C., Gong, J., et al. (2023). Next-generation immunotherapy for hepatocellular carcinoma: Mechanisms of resistance and novel treatment approaches. Cancers, 17(2), 236.

Yang, W., Li, Y., Huang, M., et al. (2024). Emerging epigenetic and metabolic mechanisms underlying drug resistance in HCC. Liver Cancer, 13(1), 101–118.

Barrett, T., Wilhite, S.E., Ledoux, P., et al. (2013). NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Research, 41(D1), D991–D995.

Jiang, W., Liu, P., Huang, J., et al. (2016). MicroRNA-34a regulates doxorubicin-induced cardiotoxicity in rats. American Journal of Translational Research, 8(2), 1295–1307.

Zhou, M., Liu, Z., Zhao, Y., et al. (2018). MicroRNA-34a enhances sensitivity to Cisplatin in human hepatocellular carcinoma cells through targeting Netrin-1. Oncology Reports, 40(1), 121–130.

Mross, K., Frost, A., Steinbild, S., et al. (2012). Phase I dose-escalation study of regorafenib in patients with advanced solid tumors. Investigational New Drugs, 30(5), 1842–1849.

Zhou, G., Soufan, O., Ewald, J., et al. (2019). NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Research, 47(W1), W234–W241.

O'Reilly, K.E., Rojo, F., She, Q.B., et al. (2006). mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Research, 66(3), 1500–1508.

Chen, W., Wu, W., Wang, Y., et al. (2020). CDHR2 suppresses tumor growth in hepatocellular carcinoma through inhibition of β-catenin signaling. Journal of Experimental & Clinical Cancer Research, 39, 140.

Wang, L., Liu, H., Li, X., et al. (2021). IL13RA2 regulates invasion and metastasis in hepatocellular carcinoma through the ERK pathway. Cell Death & Disease, 12, 733.

Liu, Z., Yang, L., Wang, J., et al. (2022). TRIB1 regulates p53 suppression and epithelial-mesenchymal transition in cancer progression. Molecular Oncology, 16(2), 459–474.

Nguyen, N.T., Yan, Y., Fei, W., et al. (2020). SNAT2-mediated amino acid transport regulates the mTOR signaling pathway in hepatocellular carcinoma. Cellular Signalling, 71, 109598.

Sun, X., Zhang, L., Liu, Y., et al. (2022). GLTPD2 regulates lipid metabolism and may affect tumor progression in HCC. Lipids in Health and Disease, 21, 9.

Fang, Y., Gao, J., Wang, J., et al. (2022). ACADS suppresses hepatocellular carcinoma via β-catenin signaling inhibition. Frontiers in Oncology, 12, 879954.

Zhang, Q., Zhou, Y., Wang, X., et al. (2021). IGFBP3 inhibits proliferation and promotes apoptosis in hepatocellular carcinoma via IGF-independent mechanisms. Oncology Reports, 45(5), 117.

He, S., Zhang, M., Zhang, Y., et al. (2023). KANK4 modulates endothelial VEGFR2 signaling and promotes arteriogenesis. Journal of Molecular Cell Biology, 15(2), 125–137.

LIST OF PUBLICATIONS AND THEIR PROOFS

Using Gene-miRNA Networks analysis to identify possibilities in currently available medications for Chronic Hepatitis-Induced HCC. Inbox ×

scopus scie <icaemconference@gmail.com> Sun, Apr 13, 12:02 AM

to me

Dear Author/s,

We are happy to inform you that your paper, submitted for the ICAEM 2025 conference has been **Accepted** based on the recommendations provided by the Technical Review Committee. By this mail you are requested to proceed with Registration for the Conference. Most notable is that the Conference must be registered on or before **APRIL 15th, 2025 from the date of acceptance.**

* Refer page No 9 in Registration form (refer attachment)

https://icaem.in/

Kindly fill the **Registration form, Declaration form (journal details)** which is attached with the mail and it should reach us on above mentioned days.

PLAGIARISM VERIFICATION

Title of the Thesis “Using Gene-miRNA Networks analysis to identify possibilities in currently available medications for Chronic Hepatitis-Induced HCC” Total Pages 46 Name of the Scholar kaushiki (23/MSCBIO/25).

Supervisor

Dr. Asmita Das

Associate Professor

Department of Biotechnology

This is to report that the above thesis was scanned for similarity detection. Process and outcome is given below:

Software used: Turnitin, Similarity Index: 5%,

Date: _____28/05/25_____

Candidate's Signature

Signature of Supervisor

5% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Cited Text
- Small Matches (less than 10 words)

Match Groups

18 Not Cited or Quoted 5%
Matches with neither in-text citation nor quotation marks

0 Missing Quotations 0%
Matches that are still very similar to source material

0 Missing Citation 0%
Matches that have quotation marks, but no in-text citation

0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

Top Sources

3% Internet sources
2% Publications
3% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.