3rd International Conference on Chemo and BioInformatics
Kragujevac, September 25-26, 2025, Serbia

ICCBIKG 2025
International Conference
on Chemo and BioInformatics

Book of Proceedings

ORGANIZERS AND SPONSORS

Republic of Serbia
MINISTRY OF SCIENCE,
TECHNOLOGICAL DEVELOPMENT AND INNOVATION

3rd International Conference on Chemo and BioInformatics

ICCBIKG 2025

BOOK OF PROCEEDINGS

September 25-26, 2025

Kragujevac, Serbia

Sponsored by

3rd International Conference on Chemo and BioInformatics, Kragujevac, September 25-26, 2025, Serbia.

Editors:

Dr. Igor Saveljić

Prof. Dr. Nenad Filipović

Technical Editors:

Dr. Vladimir Simić

Dr. Marko Antonijević

Proofreading:

Kristina Milisavljević

Press:

„Grafo Ink“, Kragujevac

Impression:

10 copies

Publisher:

Institute for Information Technologies, University of Kragujevac, Serbia, Jovana Cvijića bb, 2025

CIP - Каталогизација у публикацији Народна библиотека Србије, Београд

54:004(048)(0.034.2)
57+61]:004(082)(0.034.2)

INTERNATIONAL Conference on Chemo and BioInformatics (3 ; 2025 ; Kragujevac)
Book of Proceedings [Elektronski izvor] / 3rd International Conference on Chemo and BioInformatics, ICCBIKG 2025, September 25-26, 2025, Kragujevac, Serbia ; [editors Igor Saveljić, Nenad Filipović]. - Kragujevac : University, Institute for Information Technologies, 2025 (Kragujevac : Grafo Ink). - 1 USB fleš memorija ; 1 x 2 x 3 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 10. - Bibliografija uz svaki rad.

ISBN 978-86-82172-05-5

a) Хемија -- Информациона технологија -- Зборници b) Биомедицина -- Информациона технологија -- Зборници

COBISS.SR-ID 176312073

Committees

International Organizing Committee:

General Chairman: Dr. Igor Saveljić (Serbia)
Vice-chairmans: Dr. Nenad Janković (Serbia)
Prof. Dr. Urban Bren (Slovenia)

Members:

Dr Dejan Milenković (Serbia), Dr. Marijana Petković (Serbia), Dr. Marija Stanić (Serbia), Dr. Melina Kalagasidis Krušić (Serbia), Dr. Suzana Jovanović-Šanta (Serbia), Prof. Dr. Vladimir Ranković (Serbia), Dr. Marina Soković (Serbia), Dr. Mirjana Mihailović (Serbia), Prof. Dr. Vladimir Janjić (Serbia)

International Scientific Committee:

Chairman: Prof. Dr Nenad Filipović (Serbia)
Vice-chairmans: Academician Miloš Kojić (Serbia)
Academician Miloš Đuran (Serbia)
Prof. Dr. Zoran Marković (Serbia)
Prof. Dr. Xiaoping Chen (China)

Members:

Prof. Dr. Iztok Turel (Slovenia), Prof. Dr. Bakhtiyor Rasulev (USA), Prof. Dr. Ivan Gutman (Serbia), Prof. Nenad Kostić (USA), Prof. Dr. Demetrio Milea (Italy), Prof. Dr. Quan Yuan (China), Prof. Caroline Da Ros Montes D'Oca (Brazil), Dr. Danijela Bakarić (Croatia), Prof. Dr. Carlos Silva Lopez (Spain), Prof. Dr. Ferda Ari (Turkey), Prof. Dr. Miljan Milošević (Serbia), Dr. Edina Avdović (Serbia), Prof. Dr. Snežana Zarić (Serbia), Prof. Dr. Suzana Jovanović Šanta (Serbia), Dr. Željko Čupić (Serbia), Aleksandar Nešović (Serbia), Dr. Ana Rilak Simović (Serbia), Dr. Marko Živanović (Serbia), Dr. Jelena Katanić Stanković (Serbia), Dr. Milica Nikolić (Serbia), Dr. Dušica Simijonović (Serbia), Dr. Aleksandar Radaković (Serbia), Dr. Jasmina Obradović (Serbia), Dr. Dalibor Nikolić (Serbia), Dr. Jelena Đorović Jovanović (Serbia), Dr. Žiko Milanović (Serbia), Dr. Dejan Milenković (Serbia), Dr. Sonia Morya (India), Dr. Snežana Papović (Serbia), Prof. Dr. Biljana Ljujić (Serbia)

Local Executive Committee:

Chairman: Dr. Darko Ašanin (Serbia)
Vice-chairmans: Dr. Marko Antonijević (Serbia)
Dr. Vladimir Simić (Serbia)

Members:

Jelena Živković (Serbia), Bogdan Milićević (Serbia), Lazar Kostić (Serbia), Petar Stanić (Serbia), Aleksandra Marković (Serbia), Tamara Mladenović (Serbia), Aleksandar Nešović (Serbia), Emilija Milović (Serbia), Jovana Matić (Serbia), Nevena Milivojević (Serbia), Danijela Stojković (Serbia), Marija Branković (Serbia), Jana Baščarević (Serbia), Aleksandar Nikolić (Serbia), Lazar Milović (Serbia), Dr. Mirjana Grujović (Serbia), Dr. Katarina Marković (Serbia).

Table of contents

Identification of potential microRNA biomarkers for early diagnosis of hepatocellular carcinoma applying bioinformatics approaches

Sanja Goč[1*], Zorana Dobrijević[2], Tamara Janković[1], Filip Janjić[1], Ninoslav Mitić[1], Miroslava Janković[1]

[1]University of Belgrade, Institute for the Application of Nuclear Energy, INEP, Department of Immunochemistry and Glycobiology, Belgrade, Serbia, sanjagoc@inep.co.rs
[2]University of Belgrade, Institute for the Application of Nuclear Energy, INEP, Department of Metabolism, Belgrade, Serbia

[*]*Corresponding author*
DOI: 10.46793/ICCBIKG25.288G

Abstract: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, largely due to limited tools for early detection and the asymptomatic nature of early-stage disease. Circulating microRNAs (miRNAs) have emerged as promising non-invasive biomarkers because of their high stability in body fluids and disease-specific expression patterns. In this study, we performed an *in silico* analysis of serum miRNA expression profiles using the Gene Expression Omnibus (GEO) dataset GSE113740, which included 40 patients with HCC and 10 healthy controls. A total of 257 differentially expressed miRNAs were identified, of which seven (hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, hsa-miR-221-3p, hsa-miR-561-3p, hsa-miR-124-3p, and hsa-miR-637) were prioritized based on network metrics, including the number of single-line regulators (NSR) and transcription factor percentage (TFR) ($p < 0.05$). The majority have previously been reported as functionally associated with HCC. Meta-profile analysis across 40 cancer types revealed distinct liver cancer-specific expression for five of these miRNAs (hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, has-miR-221-3p, and has-miR-124-3p). Network topology and pathway enrichment highlighted key regulatory hubs and cancer-related targets. Ultimately, four miRNAs (hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, and hsa-miR-124-3p) emerged as promising diagnostic biomarkers for early HCC detection.

Keywords: hepatocellular carcinoma, microRNA, biomarker

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, originating from hepatocytes and frequently associated with chronic liver disease and cirrhosis . It ranks as the sixth most common cancer and the third leading cause of cancer-related deaths globally, underscoring the urgent need for reliable early diagnostic biomarkers [1]. Current surveillance methods, including serological tests and abdominal ultrasound, suffer from limited sensitivity and specificity, often missing early-stage

cases [2]. MicroRNAs (miRNAs), small, non-coding RNAs that regulate gene expression post-transcriptionally by binding to target mRNA 3′ untranslated regions (3′ UTRs), causing translation inhibition or mRNA degradation have emerged as promising biomarkers due to their stability and detectability in body fluids, alongside disease-reflective expression profiles [3].

2. Methodology

2.1. Microarray dataset selection

The GEO database (http://www.ncbi.nlm.nih.gov/geo/) was searched using keywords "hepatocellular carcinoma," "HCC," and "microRNA/miRNA/miR," yielding 371 datasets. Filtering for "*Homo sapiens*" and "serum" narrowed this to 164 datasets. Further limiting to studies with over 50 samples resulted in 32 datasets. After excluding pre-/post-operative samples and incomplete data, GSE113740 was selected for analysis.

2.2. Identification of differentially expressed miRNAs (DEMs)

DEMs between HCC and controls were identified using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) with quantile normalization. Significant DEMs were defined by $|\log_2 \text{fold change}| \geq 1$ and adjusted p-value < 0.01.

2.3. Biomarker prediction

Candidate miRNA biomarkers were predicted with miRNA-BD, prioritizing miRNAs based on the number of single-line regulations (NSR) and transcription factor percentage (TFP) within the miRNA–mRNA regulatory network. A p-value < 0.05 was considered significant for both metrics.

2.4. Biomarker selection

2.4.1. Diagnostic potential of predicted biomarkers

Statistical analyses were conducted using GraphPad Prism 10.5.0. Differences in miRNA expression between HCC and controls were assessed by the Mann–Whitney U test and visualized as scatter plots. Significance was set at $p < 0.01$.

2.4.2. Meta-profiling heatmap

To evaluate miRNA expression across multiple cancers and refine biomarker candidates, a meta-profiling heatmap was generated using dbDEMC 2.0, integrating data from 40 cancer types (https://www.biosino.org/dbDEMC/index).

2.4.3. miRNA–miRNA interaction network

The miRNA–miRNA interaction network was constructed using miRNet2.0 with validated target genes from miRTarBase v9.0 and TarBase v9.0, including disease associations (cut-off degree = 2) (https://www.mirnet.ca/). Network topology parameters, degree and betweenness centrality, were used to identify miRNA clusters.

KEGG pathway enrichment of target genes was performed using the hypergeometric test in miRNet 2.0.

3. Results and Discussion

This study aimed to identify serum miRNA biomarkers for early HCC diagnosis. We analyzed the GEO dataset GSE113740 (40 HCC, 10 controls; platform GPL21263) and identified 257 differentially expressed miRNAs (DEMs). Network metrics (NSR and TFP) prioritized seven candidates: hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, hsa-miR-221-3p, hsa-miR-561-3p, hsa-miR-124-3p, and hsa-miR-637. Notably, some are already functionally linked to HCC [4-7]. Conversely, hsa-miR-221-3p and hsa-miR-561-3p were expressed at very low levels in this dataset and excluded from differential expression analysis. This is likely due to technical limitations of microarray sensitivity or differences in serum stability compared to tissue [8]. Five miRNAs (hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, hsa-miR-124-3p, and hsa-miR-637) showed the strongest discriminatory power between HCC and controls ($p < 0.0001$; **Fig. 1**).

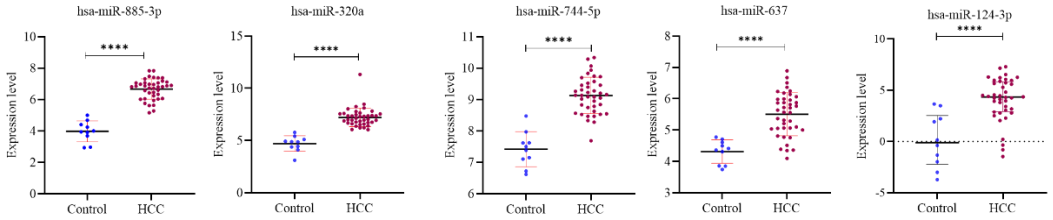

Figure 1. Expression levels of five miRNAs in HCC and control sera obtained from GEO dataset (GSE113740). HCC - hepatocellular carcinoma; **** p value < 0.0001.

Meta-profile heatmap analysis of the seven candidate miRNAs across 40 cancer types showed that five (hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, hsa-miR-221-3p, and hsa-miR-124-3p) had distinct expression patterns in HCC, each with a z-score of +4. This highlights their strong serum-based discrimination and liver cancer–specific expression, supporting their diagnostic potential and biological relevance in HCC. A miRNA–miRNA interaction network was comprised 3,081 nodes (3,074 genes and 7 miRNAs) and 10,327 edges. Topology analysis based on node degree and betweenness centrality identified four clusters: top hubs (hsa-miR-124-3p, hsa-miR-221-3p), mid-tier (hsa-miR-744-5p), medium-low (hsa-miR-885-3p, hsa-miR-320a), and peripheral (hsa-miR-561-3p, hsa-miR-637). These clusters correspond to their known roles in HCC [4-7], with cluster 4 likely representing niche or context-specific regulatory functions. Among the 3,074 gene targets (**Fig. 2A**), KEGG analysis identified 101 genes involved in cancer pathways (**Fig. 2B**). The miRNA–disease network linked has-miR-637 to multiple conditions, while hsa-miR-885-3p was specifically associated with malignant mesothelioma, a rare liver malignancy [9]. These findings suggest that hsa-miR-885-3p may assist not only in HCC detection but also in distinguishing rare hepatic malignancies, emphasizing the importance of disease-specific biomarker context. Based on differential expression, meta-heatmap profiling, and network topology, four

miRNAs: hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, and hsa-miR-124-3p, emerged as promising diagnostic candidates, either individually or within a multi-miRNA panel.

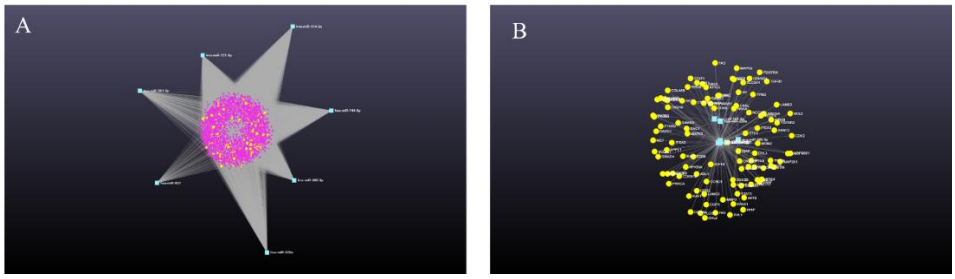

Figure 2. (A) miRNA–gene interaction network of the seven predicted miRNA biomarkers (B) Cancer-related genes extracted from the miRNA–gene interaction network.

4. Conclusion

Hsa-miR-885-3p, hsa-miR-320a, hsa-miR-744-5p, and hsa-miR-124-3p are promising HCC biomarkers with key roles in cancer development. Further validation and panel integration may improve early detection beyond alpha-fetoprotein, the most established serological biomarker in HCC diagnostics.

Acknowledgment

This research is funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Contract No: 451-03-136/2025-03/200019.

References

[1] K.S. Tummala, M. Brandt, A. Teijeiro, O. Graña, R.F. Schwabe, C. Perna, N. Djouder., Hepatocellular carcinomas originate predominantly from hepatocytes and benign lesions from hepatic progenitor cells, Cell Reports, 19 (2017) 584-600.

[2] A.E. Wang, E.A. Leven, L.T. Grinspan, A. Villanueva., *Novel biomarkers and strategies for HCC diagnosis and care*, Clinical Liver Disease (Hoboken), 23 (2024) e0152.

[3] J. Malik, M. Klammer, V. Rolny, H.L. Chan, T. Piratvisuth, T. Tanwandee, S. Thongsawat, W. Sukeepaisarnjaroen, J.I. Esteban, M. Bes, B. Köhler, M. Swiatek-de Lange., *Comprehensive evaluation of microRNA as a biomarker for the diagnosis of hepatocellular carcinoma*, World Journal of Gastroenterology, 28 (2022) 3917-3933

[4] Z. Zhang, J. Yin, J. Yang, W. Shen, C. Zhang, W. Mou, J. Luo, H. Yan, P. Sun, Y. Luo, Y. Tian, R. Xiang., *miR-885-5p suppresses hepatocellular carcinoma metastasis and inhibits Wnt/β-catenin signaling pathway*, Oncotarget, 7 (2016) 75038-75051

[5] C. Lu, Z. Liao, M. Cai, G. Zhang., *MicroRNA-320a downregulation mediates human liver cancer cell proliferation through the Wnt/β-catenin signaling pathway*, Oncology Letters, 13 (2017) 573-578

[6] W. Huang, Q. Chen, J. Dai, Y. Zhang, Y. Yi, X. Wei, Z. Wu., *miR-744-5p suppresses tumor proliferation and metastasis by targeting transforming growth factor-beta 1 (TGF-β1) in hepatocellular carcinoma (HCC)*, Journal of Gastrointestinal Oncology, 12 (2021) 1811-1822.

[7] Q. Zhao, F. Jiang, H. Zhuang, Y. Chu, F. Zhang, C. Wang., *MicroRNA miR-124-3p suppresses proliferation and epithelial-mesenchymal transition of hepatocellular carcinoma via ARRDC1 (arrestin domain containing 1)*, Bioengineered, 13 (2022) 8255-8265.

[8] T. Kupec, A. Bleilevens, S. Iborra, L. Najjari, J. Wittenborn, J. Maurer, E. Stickeler., *Stability of circulating microRNAs in serum*, PLoS One, 17 (2022) e0268958.

[9] S. Ghimire, N. Regmi, T. Yang, H. Shah, U. Srivatana, A. Sarker, H. Khan., *Primary malignant mesothelioma of the liver: Case report and review of the literature*, European Journal of Case Reports in Internal Medicine, 7 (2020) 002128