Understanding microRNAs in the context of bacterial versus viral infections

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Abstract

Introduction. MicroRNAs (miRNAs), small non-coding RNAs that regulate gene expression, have emerged as biomarkers for differentiating infection types due to their distinct expression profiles in response to pathogens. This study explores miRNA profiling using microarray technology to identify miRNA signatures that differentiate viral from bacterial infections in plasma samples.

Methods. Plasma samples were collected from patients diagnosed with either bacterial (e.g., pneumonia) or viral (e.g., human papillomavirus) infections; control samples were used to evaluate altered miRNA pattern, followed by Ingenuity Pathway Analysis (IPA) analysis.

Results. Microarray analysis revealed distinct miRNA expression patterns for bacterial and viral infections. In bacterial infections, 11 miRNAs were significantly downregulated compared to controls. Similarly, 12 miRNAs were downregulated in viral infections. Pathway analysis indicated that the altered miRNAs in bacterial infections were linked to immune and inflammatory pathways. In contrast, viral infections were associated with miRNAs involved in cellular stress and replication processes.

Conclusion. Plasma miRNA profiling offers a promising diagnostic tool to differentiate bacterial from viral infections, providing specific miRNA signatures underlying immune responses. These findings represent a foundation for developing miRNA-based diagnostics, improving the precision of infection diagnosis, and paving the way for targeted therapeutic interventions.

Keywords: plasma miRNA, microarray, bacterial infection, viral infection, biomarkers

Introduction

Despite advancements in early detection and therapeutic strategies, infectious diseases remain a significant global threat [1-3]. Major modifications appeared due to the development of advanced comprehensive technology, including microarray profiling, next-generation sequencing, vaccine development, and antimicrobial therapies. With a specificity of increase in pathogens attack and disease establishment, particularly in low and middle-income countries, pathogens, including bacteria, viruses, fungi, and parasites, are responsible for many diseases that affect populations.

Even more than the already identified and molecularly characterized pathogens, some unidentified pathogens develop due to climate changes. After their initial discovery, noncoding RNAs have received extensive attention during the last three decades due to their potentially valuable role in many diseases, including cancer, infectious diseases, immune system dysregulation, neurodegenerative diseases, and cardiovascular ones [4-6].

MicroRNAs (miRNAs) are a class of small, non-coding RNA

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molecules, typically 19-25 nucleotides in length, which regulate gene expression at the post-transcriptional level by binding to complementary sequences on target messenger RNAs (mRNAs) [6,7]. This binding results in either mRNA degradation or translational repression, thereby modulating protein expression levels. Since their discovery, miRNAs have emerged as crucial regulators in various biological processes, including cellular development, differentiation, proliferation, and apoptosis. In infectious diseases, miRNAs are pivotal in orchestrating the host immune response and pathogen-host interactions. MiRNA alterations can reflect how the host mounts a defense against invading pathogens or how pathogens manipulate host cellular pathways to evade immune responses and establish infection. For instance, viruses can encode their miRNAs or modulate host miRNA expression to inhibit antiviral responses, promote viral replication, and evade immune detection [8]. In bacterial infections, host miRNAs often regulate pro-inflammatory signaling pathways, controlling the recruitment of immune cells, the production of cytokines, and the activation of the innate and adaptive immune systems [8,9].

Given the distinct roles miRNAs play in immune regulation, profiling the expression of miRNAs during infections provides a molecular picture of the host's response to different pathogens [9-11]. This has spurred interest in using miRNA expression profiles as diagnostic biomarkers to differentiate between viral and bacterial infections. Clinically, distinguishing between viral and bacterial infections is critical, as it influences the treatment choice [9]. While bacterial infections are typically treated with antibiotics, viral infections require antiviral drugs or supportive care. Viral infections tend to induce specific miRNAs that suppress antiviral immune responses and facilitate viral persistence, whereas bacterial infections often trigger miRNAs that promote inflammation and bacterial clearance [8,12]. These distinct miRNA expression patterns serve as molecular signatures that can be used to classify the type of infection [10,13,14].

Modulating the levels of specific miRNAs through miRNA mimics or inhibitors could enhance host immune responses to infection or suppress pathogenic pathways. As the miRNA research continues to expand, it is becoming increasingly clear that miRNA profiling will play an essential role in understanding and managing infectious diseases [13-15].

Plasma miRNAs serve as promising biomarkers for various diseases, including infections, due to their stability and the ability to reflect underlying pathological processes [16,17]. These small noncoding RNA molecules play a crucial role in regulating gene expression and can be released into the bloodstream from various tissues and cells in response to disease states [12,17,18]. Their levels in plasma can provide crucial information about the presence and progression of diseases, enhancing our understanding of these complex processes [13,14,19,20].

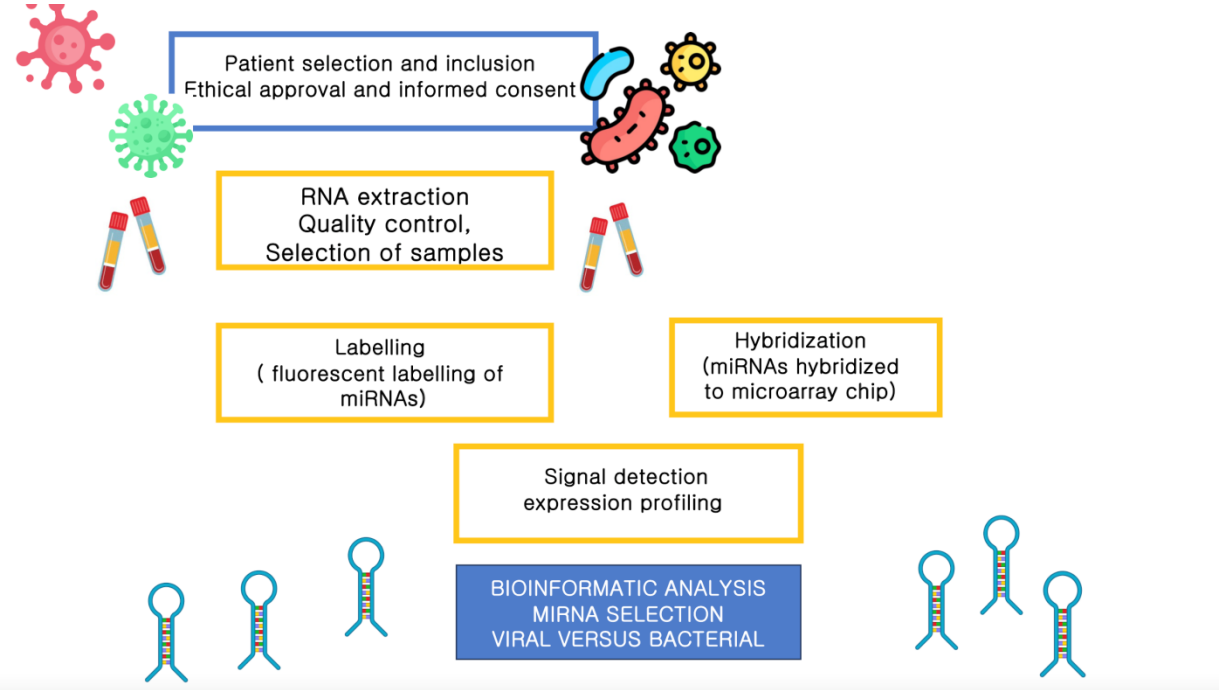

Figure 1. Experiment workflow.

Understanding plasma miRNAs in the context of bacterial versus viral infections provides valuable insights into the distinct molecular responses elicited by these pathogens [10,21,22]. By analyzing the expression profiles of plasma miRNAs using microarray (Agilent technology), we expect to identify specific miRNA signatures that distinguish between bacterial and viral infections, with the experimental workflow described in figure 1. This differentiation not only aids in diagnosing the type of infection and understanding disease mechanisms but also has the potential to revolutionize the field by leading to improved diagnostic tools and therapeutic approaches. Additionally, examining how miRNAs interact with cellular pathways and regulatory networks in response to bacterial or viral challenges enhances our knowledge of the immune response and pathogen strategies, potentially leading to these groundbreaking advancements.

Methods

Samples collection of infectious disease patients and plasma procurement

The selection criteria for our study were the presence of a viral or bacterial infection and access to plasma. We included plasma samples from patients diagnosed with viral or bacterial infections for whom informed consent was obtained, and control samples from the Biobank. The present study was approved by the institutional Ethical Committee of Leon Daniello Pulmonology Hospital, Cluj-Napoca, Romania, no 264/26.06.2018 and the Ethical Committee of Infectious Diseases Hospital, Cluj-Napoca, Romania, no 15126/21.08.2024. All plasma samples were obtained during hospitalization, then frozen and stored at −80 °C. Clinical information was collected for all patients (Table I). Informed consent was obtained from all patients before using any experimental methods or protocols, and all test protocols were under the guidelines of the hospital's and university's ethics committees.

Table I. Plasma samples included in the microarray study.

No	Diagnostic	Sample type	Type of infection
1	Pneumonia	Plasma	Bacterial
2	Pneumonia	Plasma	Bacterial
3	Pneumonia	Plasma	Bacterial
4	Human papilloma virus	Plasma	Viral
5	Human papilloma virus	Plasma	Viral
6	Human papilloma virus	Plasma	Viral
7	Control	Plasma	-
8	Control	Plasma	-

Evaluation of altered miRNA patterns

The altered miRNA profiles were analyzed using 100 ng of total RNA per sample, extracted from plasma using Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format). Sample hybridization employed the Agilent microRNA Spike-In kit, with labeling performed using the miRNA Complete Labeling and Hyb Kit. To reduce artifact risk, a purification step was incorporated using Micro Bio-Spin 6 spin columns from Biorad, followed by vacuum centrifugation for desiccation and resuspension of the pellet in 18 µL of RNase-free, microbiologically pure water.

Microarray profiling using Agilent technology platform

Hybridization was carried out according to the manufacturer's instructions, with slides (Agilent SurePrint Human miRNA v21.0 microarray, G4872A) placed in a hybridization oven at 55° C for 20 hours. Post-hybridization, the slides were washed and scanned with an Agilent Microarray Scanner. The resulting images were analyzed using Agilent Feature Extraction software to convert them into numerical expression values. Data normalization was performed using the quantile algorithm in the Agilent GeneSpring GX program; differentially expressed miRNAs were identified using the "Filter on Volcano Plot" analysis and unpaired t-test, applying a fold change threshold of 1.5 and a corrected p-value <0.05 (Benjamini-Hochberg) for comparisons between viral or bacterial infection versus control samples.

Ingenuity Pathway Analysis (IPA) of the altered miRNA profile

The differentially expressed plasma miRNAs in viral and bacterial infection versus healthy controls were loaded to Ingenuity Pathway Analysis (IPA) software, designed explicitly for interpreting complex biological data. IPA enabled the examination of the biological functions and pathways associated with the differentially expressed miRNAs by mapping them to established regulatory networks. This analysis offers insights into their roles in infection-specific biological processes and immune responses. The detailed information provided by IPA helps to elucidate the molecular mechanisms underlying the miRNA expression changes observed in plasma.

Results

Identification of plasma differentially expressed miRNAs related to bacterial and viral infection

The bioinformatics analysis, considering $p < 0.05$ and the fold change value (FC) ±1.5, revealed 11 transcripts downregulated for the in plasma from bacterial infection versus control (Table I) and 12 transcripts downregulated for the in plasma from viral infection versus control (Table II).

Table II. List of miRNAs with an altered expression level in plasma from bacterial infection versus control.

miRNA	Corrected p-value	FC
hsa-miR-24-2-5p	0	-10
hsa-miR-26a-2-3p	0.002962	-13.422
hsa-miR-3167	2.2170502E-4	-10.9888
hsa-miR-3176	9.1387825E-5	-40.2192
hsa-miR-331-5p	0.01968	-22.8854
hsa-miR-431-3p	1.1901482E-5	-22.38
hsa-miR-4522	0.002968	-17.2019
hsa-miR-548b-5p	8.144242E-4	-11.7516
hsa-miR-569	0.01786	-20.6015
hsa-miR-579-3p	0.01786	-137.592
hsa-miR-767-5p	0.002822	-13.132

Table III. List of miRNAs with an altered expression level in plasma from viral infection versus control.

miRNA	Corrected p-value	FC
hsa-miR-26a-2-3p	0.002753767	-13.422
hsa-miR-3167	3.2166985E-4	-10.9888
hsa-miR-326	3.4808484E-4	-62.7199
hsa-miR-331-5p	0.019167775	-22.8854
hsa-miR-3659	3.2166985E-4	-17.1089
hsa-miR-431-3p	2.529065E-5	-22.38
hsa-miR-4522	0.002803409	-17.2019
hsa-miR-548b-5p	7.413643E-4	-11.7516
hsa-miR-548h-5p	7.413643E-4	-26.3104
hsa-miR-569	0.017251166	-20.6015
hsa-miR-613	0.00344703	13.63366
hsa-miR-665	9.82161E-4	-12.1021

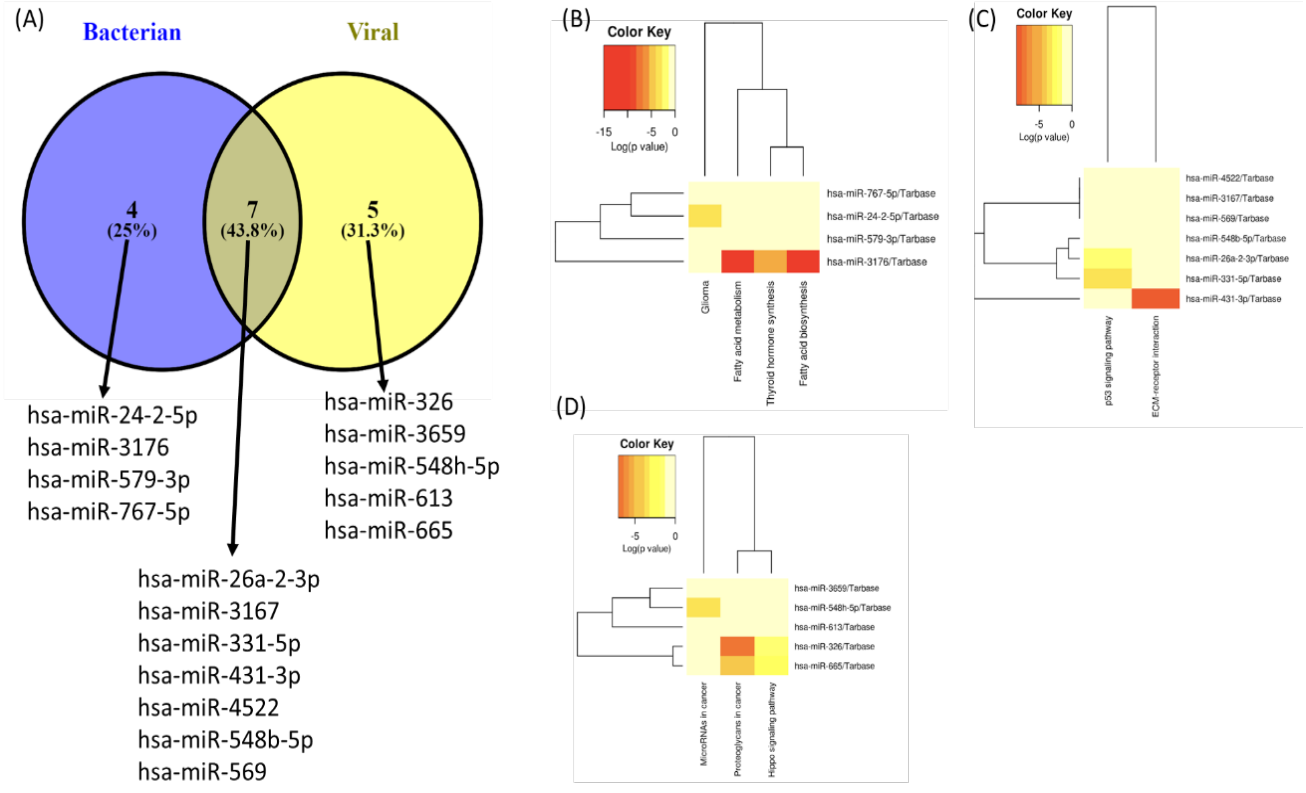

Figure 2. Altered miRNA signature in bacterial and viral plasma. (**A**) Venn diagram emphasizes common and specific miRNA signature bacterial and viral plasma; (**B**) Heatmap corresponding to altered miRNA profile specific for bacterial infection; (**C**) Heatmap corresponding to altered miRNA profile common for bacteria and viral infection; (**D**) Heatmap corresponding to altered miRNA profile specific for viral infection, generated using DIANA TOOLS - mirPath v.3. (https://dianalab.e-ce.uth.gr/html/mirpathv3/).

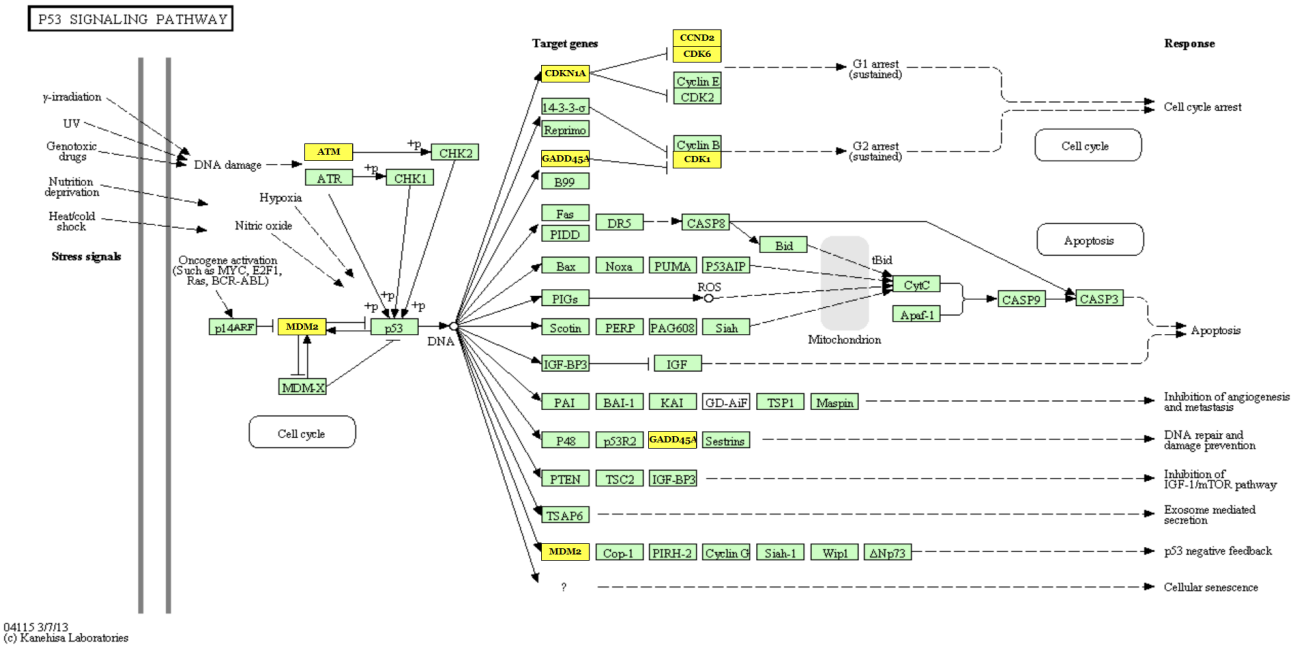

Figure S1. Analysis of the main miRNA target genes related to TP53 signalling DIANA TOOLS - mirPath v.3.

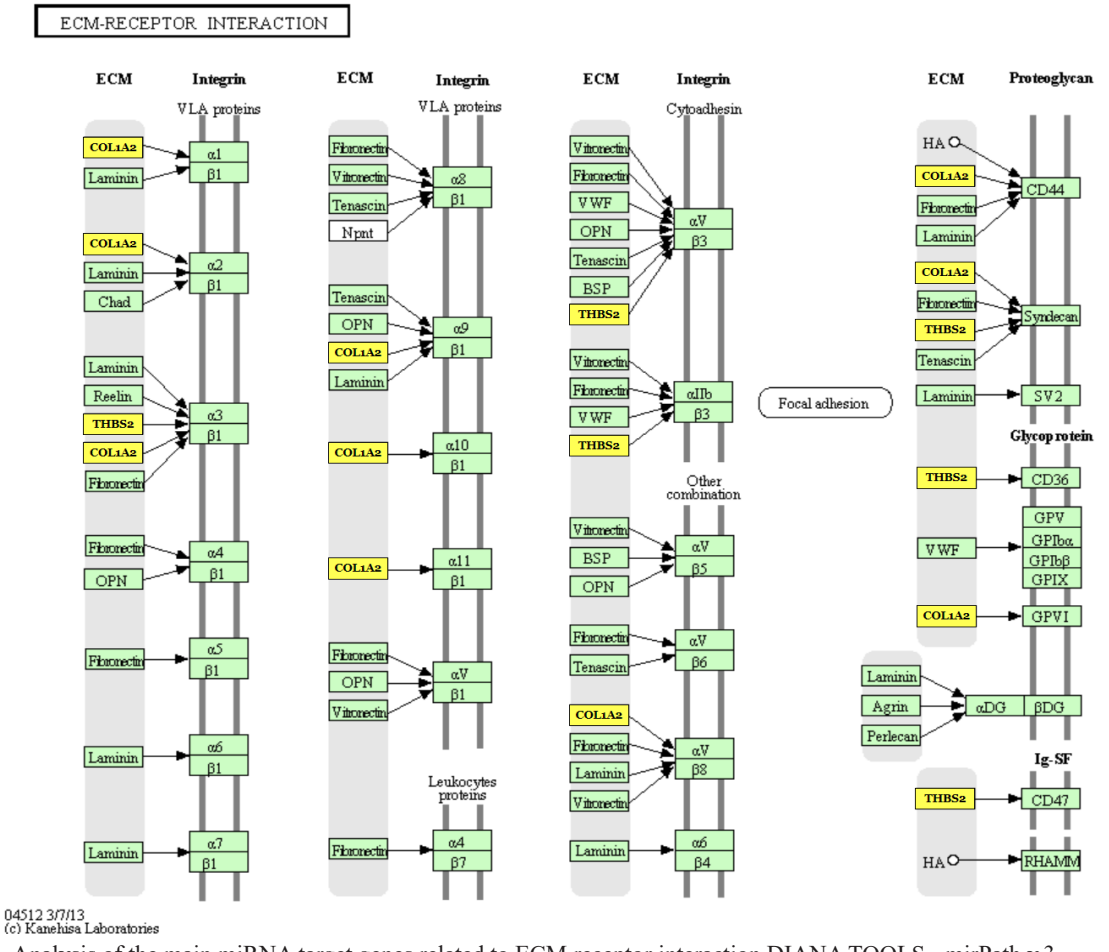

Figure S2. Analysis of the main miRNA target genes related to ECM receptor interaction DIANA TOOLS - mirPath v.3.

A Venn diagram illustrating the common and specific miRNA signatures in bacterial and viral infections in plasma would visually highlight the overlap and differences between the two types of infections (Figure 2A). Figure 2B presents a heatmap graphical representation of specific miRNAs that could provide insights into pathways and mechanisms specific to bacterial pathogens. Figure 2C is the common signature for the two infections, with emphasis on targeting important genes related to TP53 signaling (Figure S1) and ECM receptor interaction (Figure S2). Similarly, the miRNAs unique to the viral infection circle are those that are altered specifically in response to viral infections, figure 2D.

IPA analysis of the altered miRNA profile

IPA was used to evaluate the miRNA data for bacterial and viral infections; the top-associated networks and functions reveal critical insights into the molecular processes affected by each infection type. These networks are presented in table IV. These networks provide a comprehensive view of the biological functions and molecular interactions impacted by the differential expression of miRNAs in bacterial and viral infections, offering potential targets for therapeutic interventions and a deeper understanding of infection mechanisms displayed in figure 3.

Table IV. The top associated network's function generated using IPA for the analyzed two groups.

Group	Associated Network Functions	Score
Bacteria versus healthy control plasma	Gene Expression, Neurological Disease, Organismal Injury and Abnormalities	**10**
	Cancer, Cell Cycle, Cellular Development	**3**
	Organismal Injury and Abnormalities, Reproductive System Disease	**3**
	Organismal Injury and Abnormalities, Reproductive System Disease, Cancer	**3**
	Cardiovascular Disease, Heart Failure, Organismal Injury and Abnormalities	**3**
Viral versus healthy control plasma	Cellular Movement, Cancer, Organismal Injury and Abnormalities	**13**
	Cancer, Cell Cycle, Cellular Development	**3**
	Organismal Injury and Abnormalities, Reproductive System Disease	**3**
	Organismal Injury and Abnormalities, Reproductive System Disease, Cancer	**3**

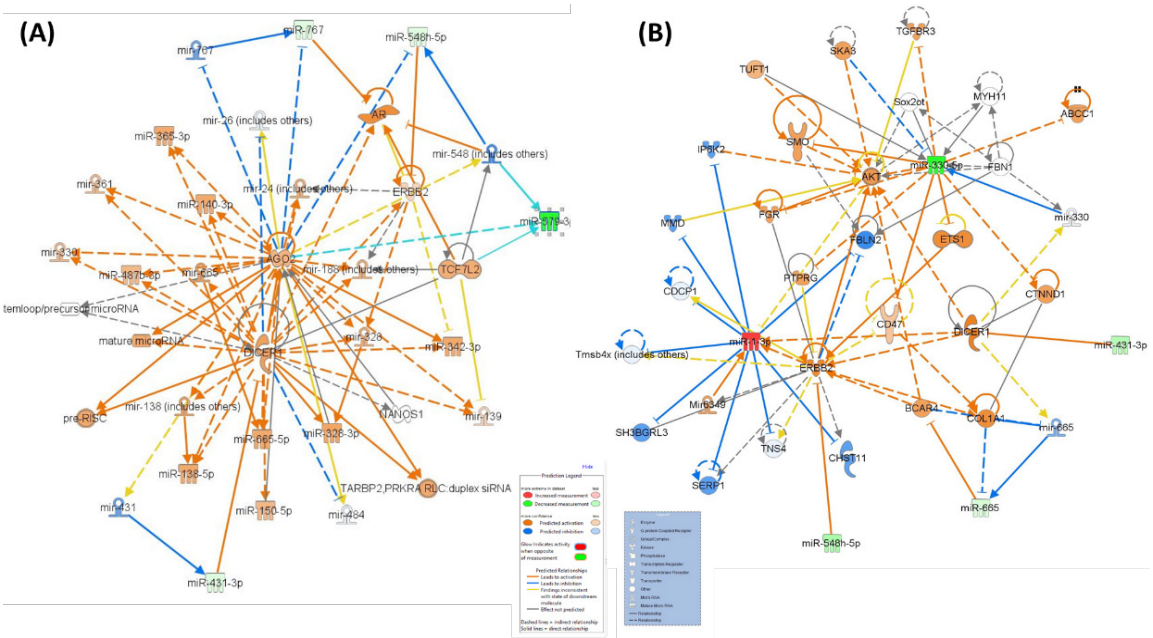

Figure 3. The miRNA-mRNA interaction network generated using IPA. (**A**) Gene Expression, Neurological Disease, Organismal Injury and Abnormalities network for Bacteria versus healthy control plasma; (**B**) Cellular Movement, Cancer, Organismal Injury and Abnormalities network Viral versus healthy control plasma. The networks are illustrated with specific color codes: downregulated miRNAs are shown in green.

Discussion

One of the major insights from this study is the potential of miRNA profiling to significantly enhance research and clinical practice in diagnosing and managing infectious diseases. By leveraging the ability to detect miRNAs in various bodily fluids, such as blood, urine, and saliva, miRNA profiling offers a noninvasive or minimally invasive method for biomarker discovery. This is especially valuable in clinical settings, where rapid, accurate, and noninvasive diagnostics are critical for managing infections. Identifying distinct miRNA expression profiles in bacterial versus viral infections opens new research avenues for exploring the underlying molecular mechanisms of pathogen-host interactions, paving the way for personalized medicine approaches enhancing treatment efficacy and minimizing side effects.

Identifying specific miRNA expression patterns in bacterial versus viral infections opens new avenues for exploring the molecular mechanisms underlying pathogen-host interactions. For example, common miRNAs could serve as general markers of infection, while specific miRNAs might help distinguish the type of pathogen, as observed in figure 2. Moreover, understanding which miRNAs are unique to each type of infection can guide the development of targeted therapies or therapeutic interventions to modulate these specific miRNAs.

These common miRNAs are likely involved in crucial regulatory pathways, such as TP53 signaling, which controls cellular stress responses, and ECM receptor interactions, which influence tissue remodeling and immune responses. By targeting these pathways, the shared miRNAs could provide insights into the fundamental mechanisms underlying both bacterial and viral infections and serve as valuable biomarkers for infection diagnosis. It also provides valuable insights into the overlapping cellular processes affected by both bacterial and viral pathogens.

The networks presented in figure 1 have a detailed overview of the biological functions and molecular interactions influenced by the differential expression of miRNAs in bacterial and viral infections. Notably, they emphasize the crucial roles of Dicer and Argonaute (Ago) proteins in bacterial infections. Dicer, an RNase III enzyme, is essential for the maturation of precursor miRNAs into functional mature miRNAs. It cleaves long double-stranded RNA and pre-miRNA molecules into smaller fragments, which are then incorporated into the RNA-induced silencing complex (RISC) [21]. Argonaute proteins, particularly Ago2, are key components of RISC that guide mature miRNAs to their target mRNAs, facilitating gene silencing through mRNA degradation or translational repression. In bacterial infections, the interplay between Dicer and Ago proteins is vital for modulating the host's immune response and controlling bacterial proliferation. Alterations in the expression or function of these proteins can impact the regulation of miRNAs that target genes involved in immune responses, inflammation, and bacterial pathogenesis [21]. By influencing the levels and activity of miRNAs, Dicer and Ago proteins help shape the host's response to bacterial infections, making them important targets for developing therapeutic strategies aimed at enhancing the immune response or mitigating inflammatory damage [21].

The role of ERBB2 and AKT in viral infections was emphasized. ERBB2, also known as HER2, is a receptor tyrosine kinase involved in cell signaling pathways that regulate cell growth, differentiation, and survival. During viral infections, ERBB2 can influence viral entry, replication, and host cell response through its involvement in signaling pathways that modulate cellular responses to stress and infection [23]. AKT, a serine/threonine kinase, is critical in several cellular processes, including metabolism, growth, and survival. In the context of viral infections, AKT signaling can be crucial for viral replication and the host cell's response to infection [24]. It regulates various downstream targets involved in anti-apoptotic pathways and immune modulation, thereby affecting the overall outcome of the infection. Dysregulation of ERBB2 and AKT pathways can impact viral pathogenesis and the host's immune response, highlighting their potential as therapeutic targets for managing viral infections and improving patient outcomes [23,24].

The present study provides the foundation for utilizing miRNAs as biomarkers in infectious diseases; further research with larger cohorts and diverse patient populations is necessary to validate these markers and refine their diagnostic potential. The main limitation is related to the small patient cohort. Consequently, further research involving larger and more diverse patient populations is essential to validate these biomarkers and enhance their diagnostic accuracy.

Conclusions

The study concludes that plasma miRNAs offer a valuable tool for distinguishing between bacterial and viral infections, with distinct miRNA expression patterns emerging as potential biomarkers for precise diagnosis. Analysis of miRNA-mRNA interaction networks revealed that these miRNAs play critical roles in regulating key biological pathways and immune responses associated with infections. Validated miRNAs show significant expression changes, enhance diagnostic accuracy, and open avenues for targeted therapeutic interventions to modulate immune responses and improve patient outcomes. These findings suggest a promising direction for future research focused on validating miRNA biomarkers and exploring their potential as therapeutic targets in diverse infection contexts.

miRNA profiling is a promising tool in the clinical management of infectious diseases, offering a new layer of molecular insight that can improve diagnosis, prognosis, and treatment personalization.

The clinical application of miRNA-based diagnostics could revolutionize infectious disease management by improving diagnostic accuracy and reducing unnecessary antibiotic use, thereby contributing to the global effort against antimicrobial resistance. With further research, technological advancements, and standardization, miRNA-based diagnostics and therapeutics could become an integral part of clinical practice, helping combat infectious diseases more effectively and reduce the misuse of antibiotics, especially in cases where the distinction between viral and bacterial infections is critical.

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