Biomolecules and Biomedicine
ISSN: 2831-0896 (Print) | ISSN: 2831-090X (Online)
Journal Impact Factor® (2024): 2.2
CiteScore® (2024): 5.2
www.biomolbiomed.com | blog.bjbms.org

SUPPLEMENTAL DATA

Salivary and urinary microRNAs as non-invasive biomarkers of minimal residual disease in pediatric acute lymphoblastic leukemia

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Full article is available at the following link: Salivary and urinary microRNAs as non-invasive biomarkers of minimal residual disease in pediatric acute lymphoblastic leukemia

Table S1. TaqMan™ Advanced miRNA Assays utilized in this study

miRNA	Mature miRNA sequence	ID	Catalog #
hsa-miR-25-3p	CAUUGCACUUGUCUCGGUCUGA	477994_mir	A25576
hsa-miR-16-5p	UAGCAGCACGUAAAUAUUGGCG	477860_mir	A25576
hsa-miR-1246	AAUGGAUUUUUGGAGCAGG	483023_mir	A25576
hsa-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA	477983_mir	A25576
hsa-miR-1290	UGGAUUUUUGGAUCAGGGA	477895_mir	A25576
cel-miR-39-3p	UCACCGGGUGUAAAUCAGCUUG	478293_mir	A25576

Table S2. Raw Cq values of the exogenous control *cel-miR-39*

Sample ID	Cq
HR054	Undetermined
HR079	Undetermined
HR104	Undetermined
HR015	*cel-miR-39* not spiked-in
HR016	*cel-miR-39* not spiked-in
HR035	*cel-miR-39* not spiked-in
SR021	*cel-miR-39* not spiked-in
SR031	*cel-miR-39* not spiked-in
SR036	*cel-miR-39* not spiked-in
HR063	29.2284946
HR062	28.295723
HR068	27.633852
HR061	27.0570164
HR103	26.0577164
HR097	26.0133762
HR047	25.7368107
SR124	25.7258072

HR071	25.6851349
SR109	25.2873325
HR098	24.836092
HR075	24.7099209
HR091	24.6954708
SR123	24.2357884
SR106	24.0189915
SR120	23.8922653
SR072	23.505003
SR040	23.4073162
SR117	23.3612976
HR087	23.3606968
HR111	23.2475643
HR046	23.1905441
SR076	22.9567242
HR056	22.8254929
SR092	22.7233028
HR083	22.7041111
HR086	22.6882534
SR084	22.6481991
HR060	22.6173859
SR122	22.1653385
HR100	21.7573547
SR088	21.6803513
SR077	21.315176
SR110	20.7487049
SR099	19.9825115
SR078	19.8831596
SR094	19.6766834
SR064	19.4527817

SR102	19.3781242
SR093	19.100399
SR057	18.1910114
SR113	17.5537014
HR118	17.0851574

Samples HR054, HR079, and HR104 were excluded from the validation phase due to insufficient amplification. The designation "*cel-miR-39* not spiked-in" indicates that the synthetic *cel-miR-39* control was unavailable during miRNA isolation. These samples contained only one aliquot, preventing RNA re-extraction for the addition of the spike-in. **Abbreviations**: HRR: High-risk of relapse; SIRR: Standard intermediate-risk of relapse.

Table S3. Mean read counts, fold change, and adjusted *p* values for differentially expressed miRNAs in saliva

miRNA	Mean read counts	Log_2 FC	Adjusted *p* value
miR-3650	142.04	-2.46	0.012
miR-4740-3p	1607.00	-2.16	0.027
miR-4713-3p	422.46	-2.13	0.022
miR-6778-5p	609.84	-2.02	0.016
miR-503-5p	2158.64	-2.00	0.016
miR-4515	1017.73	-1.79	0.047
miR-6720-3p	253.71	-1.71	0.042
miR-6817-5p	169.30	-1.68	0.022
miR-3065-5p	85.82	1.52	0.022
miR-6759-5p	136.55	1.58	0.022
miR-30e-3p	170.99	1.72	0.016
miR-200c-3p	5004.97	2.37	0.021
miR-26b-5p	399.53	2.55	0.012
miR-191-5p	4033.80	2.67	0.022
let-7d-5p	392.84	2.70	0.014

let-7i-5p	2197.45	2.78	0.027
miR-26a-5p	4528.74	2.79	0.027
miR-423-5p	4924.59	2.81	0.015
miR-375-3p	5629.12	2.88	0.019
miR-143-3p	366.56	2.94	0.010
let-7b-5p	15309.27	2.96	0.038
miR-183-5p	3376.58	2.96	0.027
let-7a-5p	16815.26	3.16	0.012
let-7f-5p	6506.01	3.19	0.012
miR-142-3p	645.15	3.22	0.002
let-7g-5p	2253.09	3.43	0.010
let-7c-5p	9101.65	3.47	0.010
miR-1290	14553.72	3.73	0.022
miR-223-3p	11637.50	3.95	0.002
miR-1246	20560.43	4.00	0.022

Abbreviation: FC: Fold change.

Table S4. Mean read counts, fold change, and adjusted *p* values for differentially expressed miRNAs in urine

miRNA	**Mean read counts**	**Log_2 FC**	**Adjusted *p* value**
miR-3162-3p	443.11	-1.70	0.027
miR-6819-3p	223.70	-1.51	0.027

Abbreviation: FC: Fold change.

Table S5. M values for miRNAs amplified by RT-qPCR

miRNA	M value
miR-1290	4.04
miR-1246	3.60
miR-223-3p	2.30
miR-16-5p	1.88
miR-25-3p	1.88

Table S6. ROC analysis and diagnostic performance metrics of individual miRNAs and the combined 3-miRNA panel, excluding the three pairs of samples from the exploratory phase

miRNA	Original AUC	95% CI	*p* value	Optimism	Corrected AUC	95% CI	Youden index	Sensitivity	95% CI	Specificity	95% CI
Combined 3-miRNA panel	0.67	0.51-0.84	0.043	0.035	0.64	0.50-0.82	0.41	0.85	0.65-1.00	0.52	0.30-0.74
miR-1290	0.60	0.42-0.77	0.292	0.008	0.59	0.44-0.76	0.76	0.70	0.50-0.90	0.65	0.43-0.83
miR-223-3p	0.64	0.47-0.82	0.109	-0.003	0.65	0.48-0.80	-0.35	0.70	0.50-0.90	0.65	0.43-0.83
miR-1246	0.55	0.37-0.73	0.585	0.023	0.53	0.41-0.69	-1.49	0.60	0.40-0.85	0.57	0.35-0.74

For single-marker models, optimal cut-off values were derived directly from the ΔCq distributions. For the combined 3-miRNA panel, the cut-off was based on the predicted probabilities from a multivariable logistic regression model integrating the ΔCq values of all three miRNAs. Optimism was estimated using bootstrap internal validation (1,000 resamples), and corrected AUC values were obtained by subtracting the estimated optimism from the original AUC. Sensitivity and specificity were calculated at the optimal cut-off defined by the Youden index.

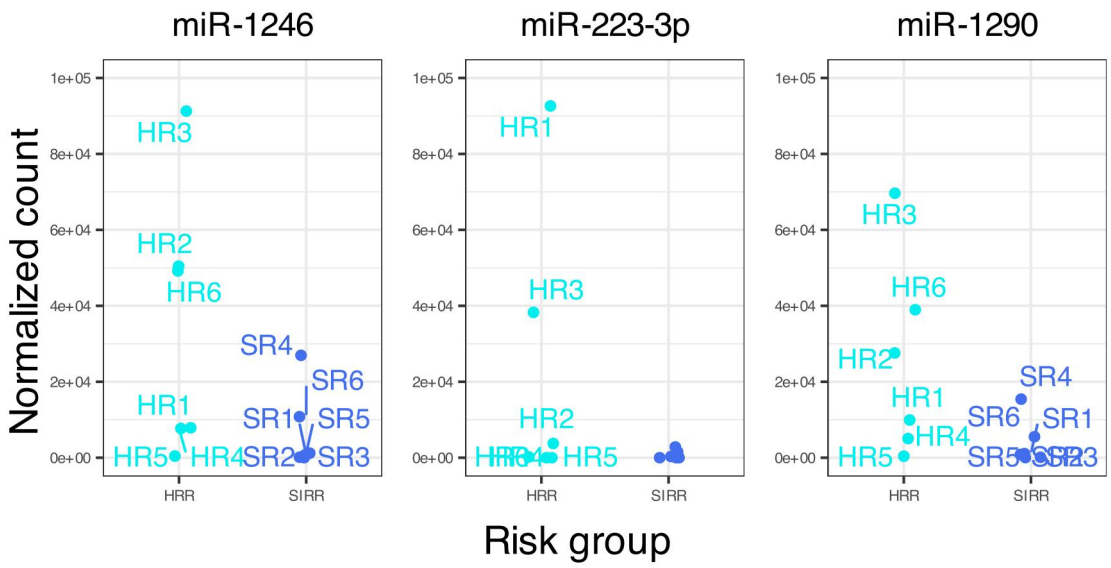


Figure S1. Normalized small RNA sequencing counts for the three most upregulated salivary miRNAs in the discovery phase. DESeq2-normalized read counts are shown for miR-1246, miR-223-3p, and miR-1290 across individual patients stratified as HRR or SIRR. Each dot represents one sample, and labels indicate sample identifiers within each risk group. The plots illustrate the markedly higher abundance of these miRNAs in multiple HRR samples relative to SIRR, supporting their prioritization for RT-qPCR validation. **Abbreviations**: DESeq2: Differential expression analysis package for RNA sequencing data; HRR: High-risk of relapse; RT-qPCR: Reverse transcription quantitative polymerase chain reaction; SIRR: Standard/intermediate-risk of relapse.

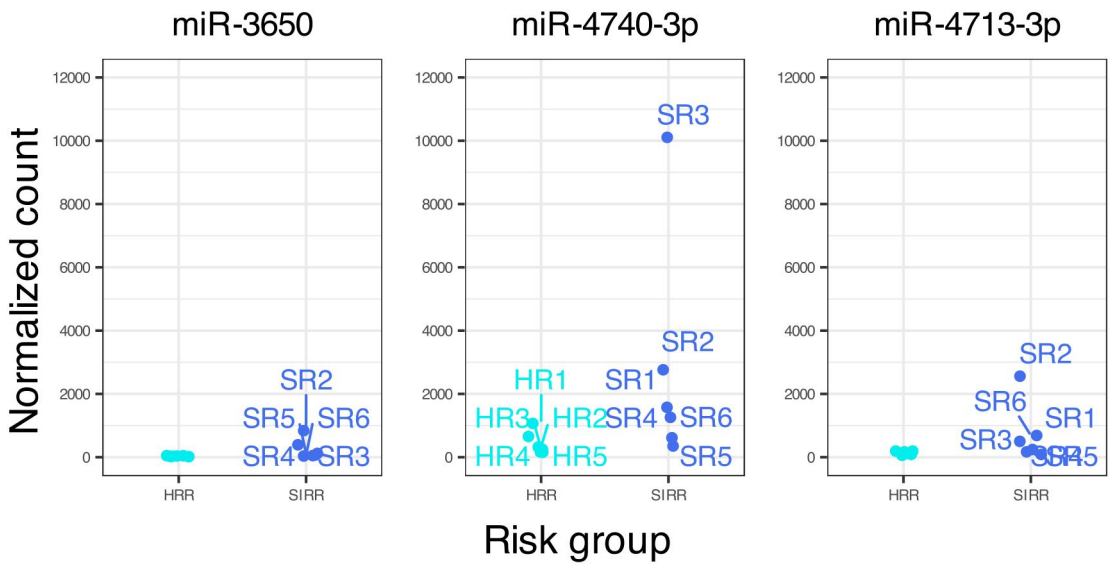


Figure S2. Normalized small RNA sequencing counts for the three most downregulated salivary miRNAs in the discovery phase. DESeq2-normalized read counts are shown for miR-3650, miR-4740-3p, and miR-4713-3p across individual patients classified as HRR or SIRR. Each dot represents one sample, and labels indicate sample identifiers within each risk group. Despite being identified as downregulated by differential expression analysis, these miRNAs showed limited separation in normalized counts between risk groups, supporting their exclusion from downstream RT-qPCR validation. **Abbreviations**: DESeq2: Differential expression analysis package for RNA sequencing data; HRR: High-risk of relapse; RT-qPCR: Reverse transcription quantitative polymerase chain reaction; SIRR: Standard/intermediate-risk of relapse.

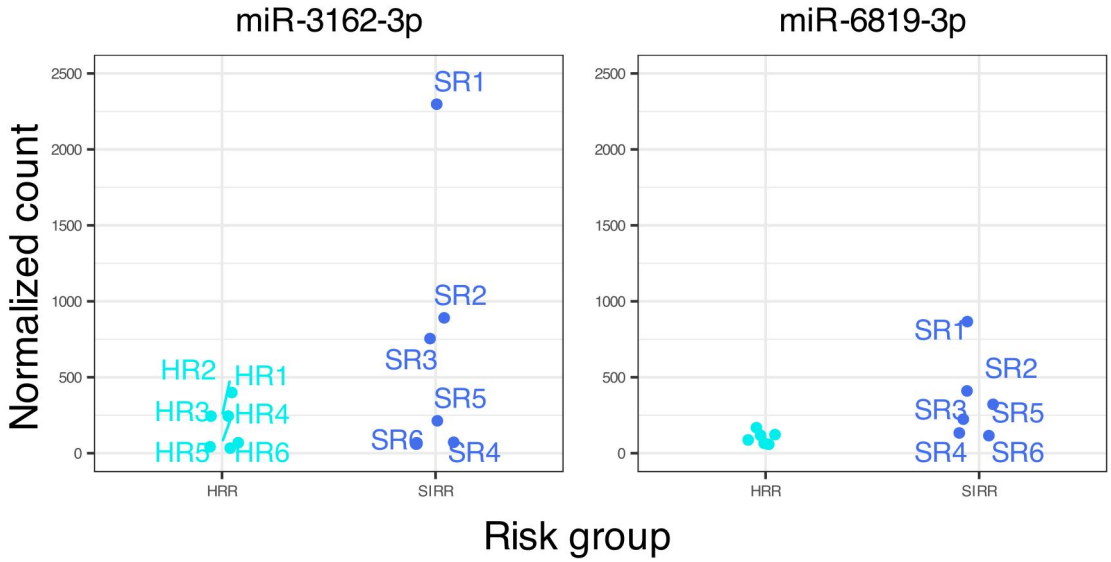


Figure S3. Normalized small RNA sequencing counts for downregulated urinary miRNAs in the discovery phase. DESeq2-normalized read counts are shown for the two urinary miRNAs identified as downregulated between risk groups (miR-3162-3p and miR-6819-3p). Each dot represents one sample, and labels indicate sample identifiers within each risk group (HRR or SIRR). Consistent with the study's selection criteria, these miRNAs showed limited separation in normalized counts between HRR and SIRR, supporting their exclusion from downstream RT-qPCR validation. **Abbreviations**: DESeq2: Differential expression analysis package for RNA sequencing data; HRR: High-risk of relapse; RT-qPCR: Reverse transcription quantitative polymerase chain reaction; SIRR: Standard/intermediate-risk of relapse.