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MicroRNAs as potential prognostic biomarkers in acute lymphoblastic leukemia: a systematic review, meta-analysis, and bioinformatics study

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Abstract

Background Acute lymphoblastic leukemia (ALL) is a hematologic malignancy characterized by malignant transformation of lymphoid precursor cells. ALL prognosis differs considerably, especially between pediatric patients and adult patients, with poor response to therapy in adults. MicroRNAs (miRNAs), small non-coding RNAs that regulate the expression of genes, are possible cancer biomarkers that predict cancer prognosis. This systematic review and meta-analysis evaluates miRNAs as prognostic biomarkers in ALL and extends the findings through ceRNA network and single-cell RNA seq analyses of validated target genes.

Material and methods We systematically searched PubMed, SCOPUS, and Web of Science (WOS) to identify studies that compared miRNA expression with the survival of ALL patients following the PRISMA guidelines. We reviewed these studies for their methodological quality, and hazard ratios (HRs) were extracted to examine miRNA expression and survival endpoints of overall survival (OS), disease-free survival (DFS), and relapse-free survival (RFS). We also applied single-cell RNA sequencing (scRNA-seq) and a competing endogenous RNA (ceRNA) network to study miRNA targets.

Results miR-335 showed a significant protective role with a pooled HR of 0.29 (95% CI, 0.12–0.68), miR-210 with a pooled HR of 0.22 (95% CI, 0.06–0.84), and miR-125b with a pooled HR of 0.39 (95% CI, 0.16–0.95) based on 22 examined studies involving 1974 patients. These miRNAs were correlated with improved OS, DFS, and RFS. We identified potential targets, whose functions were examined within key biological pathways through a ceRNA network. In various immune cell types, comparisons of B-ALL and T-ALL with normal cells showed that 36 and 98 target genes, respectively, were upregulated, while 21 and 19 genes were downregulated. Additionally, when comparing T-ALL to B-ALL cells, 70 target genes had increased expression, and 13 genes had decreased expression. Among all analyzed lineages, leukemic blast cells exhibited the most consistent and pronounced alterations in the expression of validated miRNA target genes, suggesting that blasts are the primary population influenced by these regulatory interactions in ALL.

Conclusion These findings support miRNAs as valuable prognostic biomarkers for ALL with potential for personalized therapy. The context-dependent role of these miRNAs highlights the need for confirmation through large,

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multicenter trials. The constructed ceRNA network provides useful insights into their regulatory mechanisms, opening new directions for therapeutic strategies.

Keywords Acute lymphoblastic leukemia (ALL), Competing endogenous RNA (ceRNA) Network, Non-coding RNAs, MicroRNAs, Single-cell RNA sequencing (scRNA-seq), Prognosis, Survival

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood, and extramedullary sites. The hallmark of ALL is chromosomal abnormalities and genetic alterations involved in the differentiation and proliferation of cells. Therefore, diagnosis relies on morphology, immunophenotype, and genetic characteristics, which differentiate malignant cells from normal progenitor cells and other neoplasms [1, 2]. Standard treatment for ALL involves multiple chemotherapeutic agents administered in phases of induction, consolidation, and maintenance over 2.5 to 3 years, along with central nervous system (CNS) prophylaxis and hematopoietic stem cell transplantation (HSCT) [3]. ALL accounts for 10% of leukemia cases in the United States, with an incidence rate of 1.6 per 100,000. The 5-year overall survival (OS) rate by treatment was 72% in 2010–2017 [4]. Although 80% of new cases occur in pediatrics, the prognosis is poor in adults. While intensive chemotherapy leads to more than 80% cure in childhood ALL, this number decreases to 40%–50% and 15% in adult and older ALL, respectively [5–7]. Ph-positive ALL was considered a separate ALL entity from 2010 with the introduction of tyrosine kinase inhibitors (TKIs). As the incidence of Ph-positive ALL increases with age, by performing an age-matched comparison, older patients with Ph-positive ALL had better outcomes due to the availability of TKIs [4]. Beyond mortality related to leukemia, survivors of hematologic malignancies also face significant long-term health problems caused by their treatments. Patients with these cancers have more than a five-fold increased risk of developing hypothyroidism and more than a two-fold increase in diabetes mellitus compared to the general population, with the highest risk seen in those exposed to chemotherapy, radiotherapy, or total body irradiation [8, 9]. In addition to biological and treatment-related factors, psychosocial comorbidities may also affect survival. A recent meta-analysis showed that depression is associated with reduced overall survival in patients with hematologic malignancies [10]. These findings emphasize the need for accurate prognostic biomarkers to guide risk-based treatment plans that aim to maximize cure rates while reducing late endocrine complications, especially in younger ALL patients.

MicroRNAs, small non-coding RNAs, consist of 17–25 nucleotides. They are produced by the RNase activity of Dicer and are involved in post-transcriptional gene expression regulation of 30% of genes by binding to the 3' untranslated region (UTR) of mRNAs using complementary interaction, inhibiting ribosomes from translating mRNAs. In neoplasms, they can be both oncogenic and gene suppressors as they activate different pathways inside the cell, including cell proliferation, differentiation, and apoptosis [11, 12]. For instance, overexpression of miR-24 decreases apoptosis in leukemic cells, contributing to impaired homeostasis and tumor progression. Molecular targets of miR-24 include human activin receptor type 1 (ALK4), proapoptotic proteins (fas-associated factor 1 (FAF-1), Bcl-2-like protein 11 (BIM), apoptotic peptidase activating factor 1 (APAF-1), and caspase 9), and cell cycle proteins. Similarly, in clinical studies, overexpression of miR-24 was associated with a lower survival rate and remission [13–15]. Another closely linked miRNA to ALL is miR-125b, which is downregulated in T-ALL and pre-B-ALL patients [16]. Overexpression of miR-125b in pre-B ALL was associated with poor prognosis and resistance to vincristine and daunorubicin treatment [17]. This microRNA promotes leukemia by targeting the tumor suppressor interferon regulatory factor 4 (IRF4) and the A+T-rich interaction domain 3 A protein (ARID3a) [18, 19].

Growing evidence indicates that miRNAs are diagnostic biomarkers, as significant differences are reported in the miRNA expression profile of ALL patients [20, 21]. For instance, a diagnostic panel of miR-128a and miR-223 has a high diagnostic odds ratio for childhood ALL when compared to AML. Overexpression of miR-128 is also associated with glucocorticoid response and survival of childhood patients [22, 23]. When T-ALL patients are compared to healthy controls, significant downregulation of miR-30, miR-24-2, and miR143-145 clusters, miR-574, and miR-618, along with significant overexpression of miR-128, miR-181, miR-130, and miR-17, is notable [21]. In studies related to the prognosis of ALL, several miRNAs are reported to be associated with its prognosis [24].

In this study, we performed a systematic review and meta-analysis to assess the miRNAs associated with ALL patients' survival outcomes. Compared to

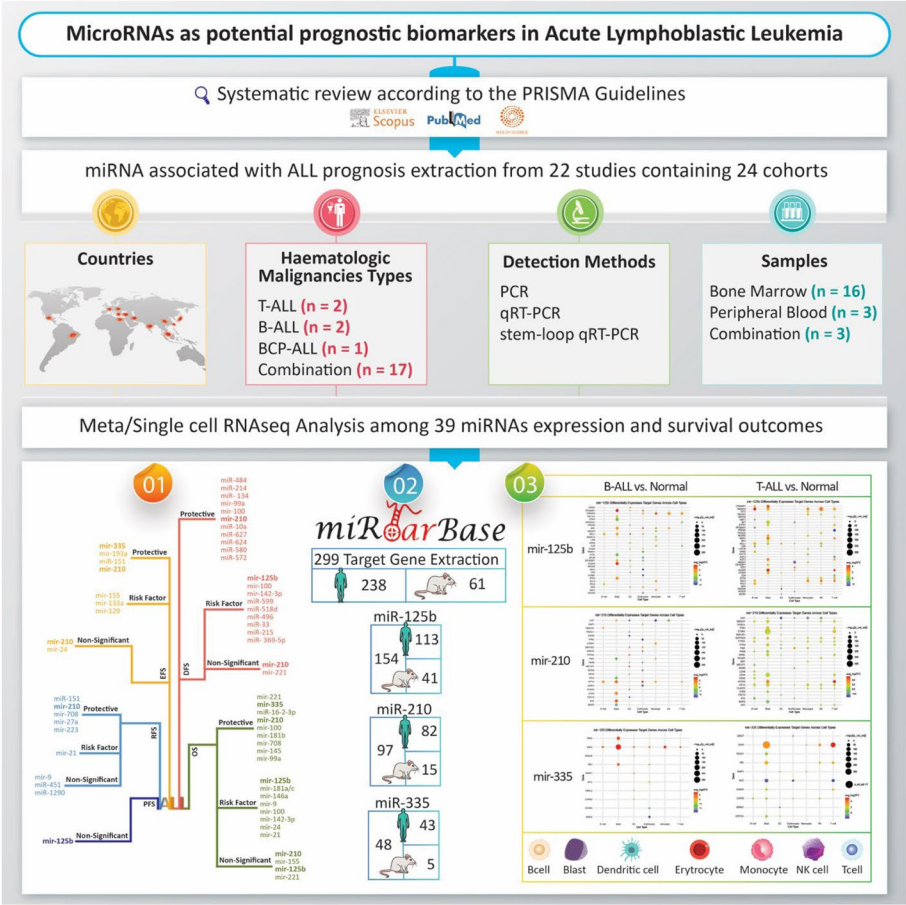


Fig. 1 Graphical abstract. A systematic literature search was conducted in PubMed, Scopus, and WOS, following the PRISMA guidelines, which resulted in 22 studies encompassing 24 cohorts that were eligible for meta-analysis. These studies analyzed miRNA expression in bone marrow and peripheral blood samples from pediatric and adult ALL patients using PCR-based detection methods. Among them, 39 miRNAs were assessed in the prognosis of ALL, with miR-335, miR-210, and miR-125b consistently associated with improved survival. The experimentally validated target genes of these miRNAs (299 in total) were extracted from miRTarBase, which includes both human and non-human genes. scRNA-seq data from T-ALL, B-ALL, and normal samples were analyzed to assess the differential expression of miRNA target genes in different immune cell subtypes, showing subtype-specific patterns of gene regulation

previous reviews of miRNAs in ALL prognosis [24, 25], which included up to 17 studies and approximately 1500–1750 patients, our work evaluated 22 studies with 1974 patients and extends the evidence base through 2023. Furthermore, the target genes of significant miRNAs were assessed using single-cell analysis (Fig. 1).

Materials and methods
Protocol and registration

This systematic review was conducted according to the PRISMA guidelines. The scientific question of the study was formulated using the PICO framework, which includes four main aspects of “Population,” “Intervention,” “Comparator,” and “Outcome.” The transparent protocol for this review is available under the registration

code CRD42024548362 on the International Prospective Register for Systematic Reviews (PROSPERO).

Objectives and scientific question
Our objective was to identify miRNAs that stratify ALL patients into prognostic risk groups. This will further improve future therapy planning, consultations, and patient outcomes.

Literature search methodology
The databases PubMed, SCOPUS, and Web of Science, known for their extensive and wide-ranging coverage, were utilized to gather pertinent papers. The search

terms included “Acute Lymphoblastic Leukemia,” “acute lymphoid leukemia,” “Acute lymphocytic Leukemia,” “Prognosis,” “survival,” “outcome,” “response,” “relapse,” and “MicroRNA.” The search strategies, along with the number of retrieved records from each database, are detailed in Supplementary File 1.

Eligibility criteria for inclusion and exclusion

Eligible studies included original research papers that investigated the ability of miRNAs to predict outcomes in a population consisting of ALL patients. There were no restrictions on patient age or publication date, ensuring the inclusion of all relevant studies up to now. Studies using animal models or commercial human or animal cell lines, non-original research such as book chapters and review articles, and papers published in languages other than English were deemed unsuitable and excluded from further review. For papers whose full text was unavailable, contact was made with the corresponding authors; those who did not respond were excluded.

Literature screening and data extraction

A two-step process was employed to review the collected documents. Initially, four reviewers independently evaluated the title and abstract of each paper against the eligibility criteria using the Rayyan web tool. Papers were categorized as “excluded” or “included” based on predefined criteria, with excluded papers marked accordingly. Next, included papers went through a second step where their full texts were independently reviewed by three reviewers, again using predefined exclusion labels. The final included studies underwent data extraction, with three reviewers extracting bibliographic information—such as study title, primary author’s name, publication year, and study design—as well as data relevant to the study’s scientific question. If disagreements persisted among reviewers at any stage, an additional reviewer was consulted to reach a consensus.

Quality assessment

We implemented the Newcastle-Ottawa Scale (NOS), the most widely used tool for assessing the methodological quality of non-randomized studies, in our meta-analysis [26, 27]. This scale aims to evaluate certain aspects of the quality of the selected studies. Each study in the review was reviewed by two evaluators, and any disagreements were resolved through discussion or by consulting a third reviewer. The highest score was nine, indicating the best quality, and the lowest was 0. In our analysis, we classified studies with scores of ≥ 7 as high or good quality and those with scores below 7 as lower quality. This threshold was selected beforehand because scores of ≥ 7

indicate that a study meets most NOS criteria across the selection, comparability, and outcome domains, and this cutoff has been widely used in oncology-focused meta-analyses of observational studies [28, 29].

Meta-analysis

A systematic meta-analysis was conducted to evaluate the association between miRNAs and survival outcomes in patients with ALL. The primary effect size measure was the Hazard Ratio (HR), which quantified the relationship between miRNA expression and survival outcomes, such as overall survival (OS), disease-free survival (DFS), event-free survival (EFS), and relapse-free survival (RFS). For studies that directly reported HRs along with their 95% confidence intervals (CIs), these values were utilized in the meta-analysis. In instances where only Kaplan-Meier (KM) survival curves were provided, HRs and CIs were extracted using the Automeris digitizer and the Guyot method for reconstruction [30, 31]. A random-effects model was employed to account for potential heterogeneity across studies, as variability in study design, patient populations, and methodologies was expected. Heterogeneity was assessed using the I^2 statistic, with values greater than 50% indicating significant heterogeneity. To explore potential sources of this heterogeneity, subgroup analyses were conducted based on the type of miRNA. Publication bias was assessed through funnel plots and Egger’s test. All statistical analyses were performed using R software (version 4.3.2) and relevant meta-analysis packages. Missing data was addressed using the aforementioned digitization methods and Guyot approach for studies lacking direct HR and CI data.

Heterogeneity investigation

We used multilevel random-effects models with crossed random effects for study and miRNA, employing metafor::rma.mv. We present pooled HRs with 95% CIs and 95% prediction intervals, and separate heterogeneity into study- and miRNA-level I^2 , using the average within-study variance as the sampling-error component. Prespecified sensitivity analyses were limited to qPCR-only, BM-only, pediatric-only, reported HR-only, region, and high-quality studies.

MiRNA target identification

To determine the regulatory impact of the selected miRNAs, their experimentally validated target genes were retrieved from the miRTarBase [32] (<https://mirtarbase.cuhk.edu.cn/>), an experimentally validated miRNA-target interaction database. Both human and non-human target genes were included for further analysis.

Single-cell RNA sequencing analysis

To investigate differentially expressed genes between normal and acute leukemia, scRNA-seq datasets from T-ALL, B-ALL, and healthy controls were retrieved from the Gene Expression Omnibus (GEO) database under accession IDs GSE154109 [33] and GSE227122 [34]. The datasets were analyzed using the Seurat R package. After quality control and SCTransform normalization, Harmony was employed to reduce batch effects and facilitate data integration (Harmony run on the top 30 principal components, using default parameters/ $\theta=2$). Dimensionality reduction techniques, such as principal component analysis (PCA) and Uniform Manifold Approximation and Projection (UMAP), were applied, with 30 dimensions used for embedding. Unsupervised clustering was performed using Seurat's FindNeighbors and FindClusters functions with a resolution parameter of 0.2, and the resulting clusters were annotated based on canonical gene markers [33, 34], yielding seven cell populations (B cells, T cells, blasts, dendritic cells (DCs), erythrocytes, monocytes, and natural killer (NK) cells). Differential expression analysis using the Wilcoxon rank-sum test identified significantly upregulated or downregulated genes ($|\log_2FC| > 1$; adjusted P -value < 0.05). Genes with significant expression changes in at least one comparison were retained for further analysis.

Results

Study identification

From the initial literature search, 856 studies were identified. After the title and abstract, 783 non-relevant studies were excluded, including meta-analyses, book chapters, reviews, in vitro/animal studies, and other unrelated publications. During the full-text review, 51 studies were removed due to reasons such as the full text being inaccessible, lack of sufficient result reporting, absence of prognostic analysis, or unavailability of KM plots. In the end, 22 studies containing 24 cohorts were found eligible, contributing 66 entries for individual prognostic miRNAs with their hazard ratio (HR) for meta-analysis. One additional study (Kaddar 2009) was unsuitable for HR extraction, as it divided patients into four groups and no HR was calculated [35]. The selection workflow is illustrated in Fig. 2.

Characteristics of included studies

The articles, published between 2010 and 2023, involved 1974 patients across four regions: East and South Asia (including China, Japan, Taiwan, Malaysia, India), the Middle East and North Africa (including Egypt, Iran, Turkey), Europe (including Greece, the Netherlands), and Latin America (including Mexico, Brazil). The location of each study was determined by the region from which

the subjects were sourced. The country of origin for each patient cohort, serving as an indicator of population region and ancestry, is presented in Table 1 under the "Country" column. The cancers studied included T-ALL ($n=2$), B-ALL ($n=2$), BCP-ALL ($n=1$), or a combination thereof ($n=17$).

Total miRNA or miRNA from purified mononuclear cells was analyzed in samples of bone marrow ($n=16$), peripheral blood ($n=3$), or a combination ($n=3$). MiRNA detection methods included microarray and various polymerase chain reaction (PCR) techniques, specifically quantitative real-time PCR (RT-qPCR) and stem-loop RT-qPCR. The median primarily served as the threshold for categorizing patients into high and low expression groups by miRNA expression. In studies involving a control group, this threshold was determined by ROC curve analysis of miRNA expression within that group. Numbers were insufficient for a cut-off-type meta-analysis ($k < 2$ per stratum). In total, 39 distinct miRNAs were analyzed, 31 of which were identified as protective factors while 21 were linked to increased risk. Conversely, 14 miRNAs showed no significant association with survival. DFS, OS, RFS, EFS, and PFS were evaluated in 1881, 1824, 822, 606, and 120 patients, respectively (Table 1).

Quality assessment

Of 23 studies, 13 (56.5%) were rated as high quality (scores ≥ 7) and 10 (43.5%) as moderate quality (scores 5–6). All studies scored well in the Selection domain. However, variability was noted in the Comparability domain, with only 10 (56.5%) adjusting for key factors. In the Outcome domain, while 10 (56.5%) adequately defined survival endpoints and follow-up durations, limited reporting on blinded assessments and handling of missing data reduced scores for some studies. The quality assessment of the included studies is presented in Supplementary File 2.

Meta-analyses for miRNAs with Multiple HRs

For the evaluation of the prognostic significance of individual miRNAs, meta-analyses were performed based on eight miRNAs, including miR-155, miR-335, miR-221, miR-125b, miR-24, miR-210, miR-100, and miR-151 that had at least two reported HRs across different cohorts. The results are shown in Fig. 3. Three miRNAs showed significant protective associations with survival outcomes. The protective effects of miR-335 ($k=2$; pooled HR, 0.29; 95% CI, 0.12–0.68; $I^2=28.2\%$) and miR-210 ($k=4$; pooled HR, 0.22; 95% CI, 0.06–0.84; $I^2=61.8\%$) were consistent with low-to-moderate heterogeneity. Additionally, miR-125b significantly protected OS ($k=2$; pooled HR, 0.39; 95% CI, 0.16–0.95; $I^2=0\%$) with no

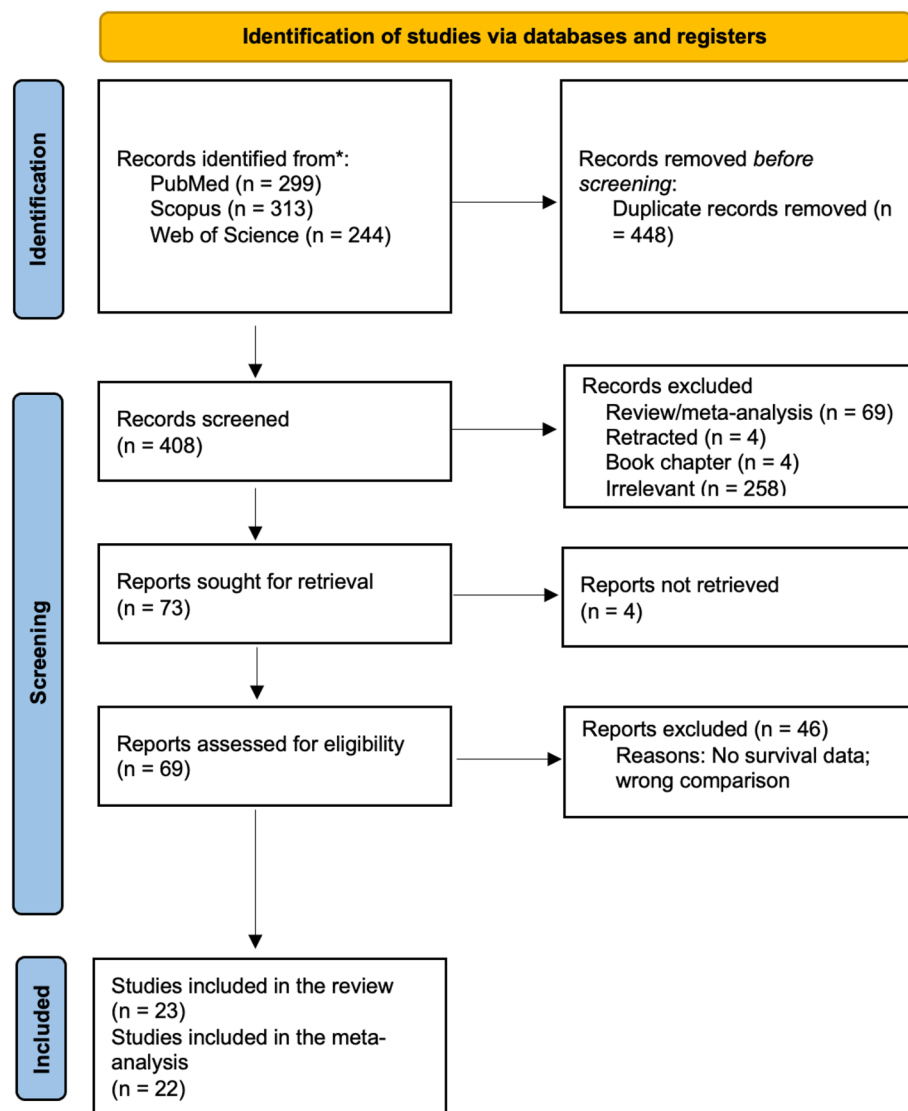


Fig. 2 PRISMA flow diagram

heterogeneity. The other miRNAs, including miR-155, miR-24, miR-221, miR-100, and miR-151, showed wide CIs, reflecting a lack of statistical significance. Overall, the results suggest that miR-335, miR-210, and miR-125b are consistently linked to better survival outcomes in ALL. The significance of other miRNAs remains uncertain due to variability and a lack of precision in the data (Supplementary File 3).

Remarkably, several miRNAs exhibited discordant or context-dependent effects across different cohorts. miR-100 showed opposite directions in two studies; miR-221 was protective in mixed-ALL but associated with risk in T-ALL; miR-210 was mostly protective, though one cohort indicated risk; miR-125b

was protective in one pediatric cohort but had no effect in another; and miR-155 was a risk marker for EFS but not for OS. For miRNAs with conflicting directions, we report estimates without making a definitive directional claim and label them as hypothesis-generating. Context-dependent miRNAs and their opposing cohort-level estimates are summarized in Table 2.

The association between miRNA expression and survival: miRNA expression is associated with OS, DFS, EFS, and RFS in ALL patients

To better understand the prognostic roles of miRNAs in ALL, we categorized the data by survival outcomes: OS, DFS, EFS, RFS, and PFS. For each outcome, we divided

Table 1 Characteristics of Included Studies

No.	First author, year	Country*	miRs	Malignancy	Age group	Sample size (female)	Follow-up (median)	Cut-off Method	Sample source	Cell type	Quantification method	Survival outcome(s)	HR source
1	Piatopoulou 2017 [16]	Greece	miR-125b	ALL	Pediatric	125 (NA)	79 m	Median	BM	Total RNA	RT-qPCR	OS; DF	multi
2	Wang 2010 [36]	China	miR-221, miR-181a/c, miR-146a	ALL	Adult	32	2 y	Median	BM	Mononuclear cells	RT-qPCR	OS	uni
3	Yan 2013 [37]	China; Malaysia; India	miR-335	ALL	Pediatric	73; 63	4.74 y	Median	BM	Mononuclear cells	RT-qPCR	OS; EFS	multi; km
4	Liang 2021 [38]	Turkey; Netherlands (GEO)	miR-155	ALL	Pediatric	42	130 m (km)	Median	BM	Total RNA	RT-qPCR	EFS	km
5	Sugita 2014 [39]	Japan	miR-9	ALL	Adult	32 (13)	838.5 days	Mean+2SD in control group	BM	Mononuclear cells	RT-qPCR	OS; RFS	multi; uni
6	Avigad 2016 [40]	Israel; Czech Republic; Netherlands	miR-451, miR-151, miR-1290	BCP-ALL	Pediatric	138	92 m	First-quartile; third-quartile	BM	Total RNA	RT-qPCR	RFS	km
7	Mei 2017 [41]	China	miR-210	ALL	Pediatric	91 (34)	9.89 ± 2.15 and 9.68 ± 2.14 in high and low wxpres-sion group respectively	Median	BM	Mononuclear cells	RT-qPCR	RFS	multi
8	Chu 2023 [42]	China	miR-16-2-3p	ALL	Pediatric	106 (56)	48 m	Median	serum	NA	RT-qPCR	OS	multi
9	Mei 2014 [43]	China	miR-210	ALL	Pediatric	76; 38	61 m	Based on ROC curve (3.8243)	BM	Total RNA	RT-qPCR	OS; DFS; EFS	km
10	Hassan 2020 [44]	Egypt	miR-100	ALL	Pediatric	85 (38)	3.5 y	Based on ROC curve (3.5)	BM	Total RNA	RT-qPCR	OS; DFS	km
11	Ly 2012 [45]	China	miR-142-3p	T-ALL	Adult	68 (25)	50 m	Median	PB	Total RNA	RT-qPCR	OS; DFS	km
12	Zhou 2017 [46]	China	miR-181b, miR-155	B-ALL	Adult	76 (36)	32 m	Median	BM	Mononuclear cells	RT-qPCR	OS	multi
13	Organista-Nava 2015 [15]	Mexico	miR-24	ALL	Pediatric	111 (41)	70 m (km)	75th percentile	BM	Total RNA	RT-qPCR	OS	km

Table 1 (continued)

No.	First author, year	Country*	miRs	Malignancy	Age group	Sample size (female)	Follow-up (median)	Cut-off Method	Sample source	Cell type	Quantification method	Survival outcome(s)	HR source
14	Schotte 2011 [17]	Netherlands	miR-10a, miR-33, miR-134, miR-214, miR-215, miR-369-5p, miR-484, miR-496, miR-518d, miR-572, miR-580, miR-599, miR-624, miR-627	ALL	Pediatric	81 (NA)	120 m (km)	Median	BM/PB	Mononuclear cells	RT-qPCR	DFS	multi
15	El-Khazragy 2019 [47]	Egypt	miR-125b	ALL	Pediatric	120 (54)	1 m-10 years	Median	PB	Mononuclear cells	RT-qPCR	OS; PFS	multi
16	Labib 2017 [48]	Egypt	miR-21	B-ALL	Pediatric	75 (34)	5 y	Upper quartile leve	BM/PB	Mononuclear cells	RT-qPCR	OS; RFS	multi
17	Han 2011 [49]	China	miR-708, miR-223, miR-27a	ALL	Pediatric	70 (NA)	38 m; 36 m	Median	BM	Total RNA	RT-qPCR	OS; RFS	multi; km
18	Chang 2022 [50]	Taiwan	miR-129, miR-133a, miR-151, miR-193a	ALL	Pediatric	60 (32)	19 y (km)	Median of risk score	BM/PB	Total RNA	stem-loop RT-qPCR	EFS	uni
19	Long 2020 [51]	China (Target database)	miR-145	ALL	Pediatric	179	12 m (km)	Median	BM	NA	miRNA-seq (TARGET database)	OS	km
20	Gimenes-Teixeira 2013 [52]	Brazil	miR-221, miR-374	T-ALL	Pediatric; Adult	48 (9)	27 m	Median of risk score	BM	Total RNA	RT-qPCR	OS; DFS	uni
21	Li 2013[53]	China	miR-100, miR-99a	ALL	Pediatric	111 (36)	5 y	NA	BM	Total RNA	RT-qPCR	OS; DFS	km
22	Esmaili 2020 [54]	Iran	miR-24	ALL	Pediatric	74 (NA)	40 m	Median	PB (plasma)	Total RNA	RT-qPCR	EFS	km

*The country of origin for each patient cohort, serving as an indicator of population region and ancestry, is presented in Table 1 under the “Country” column

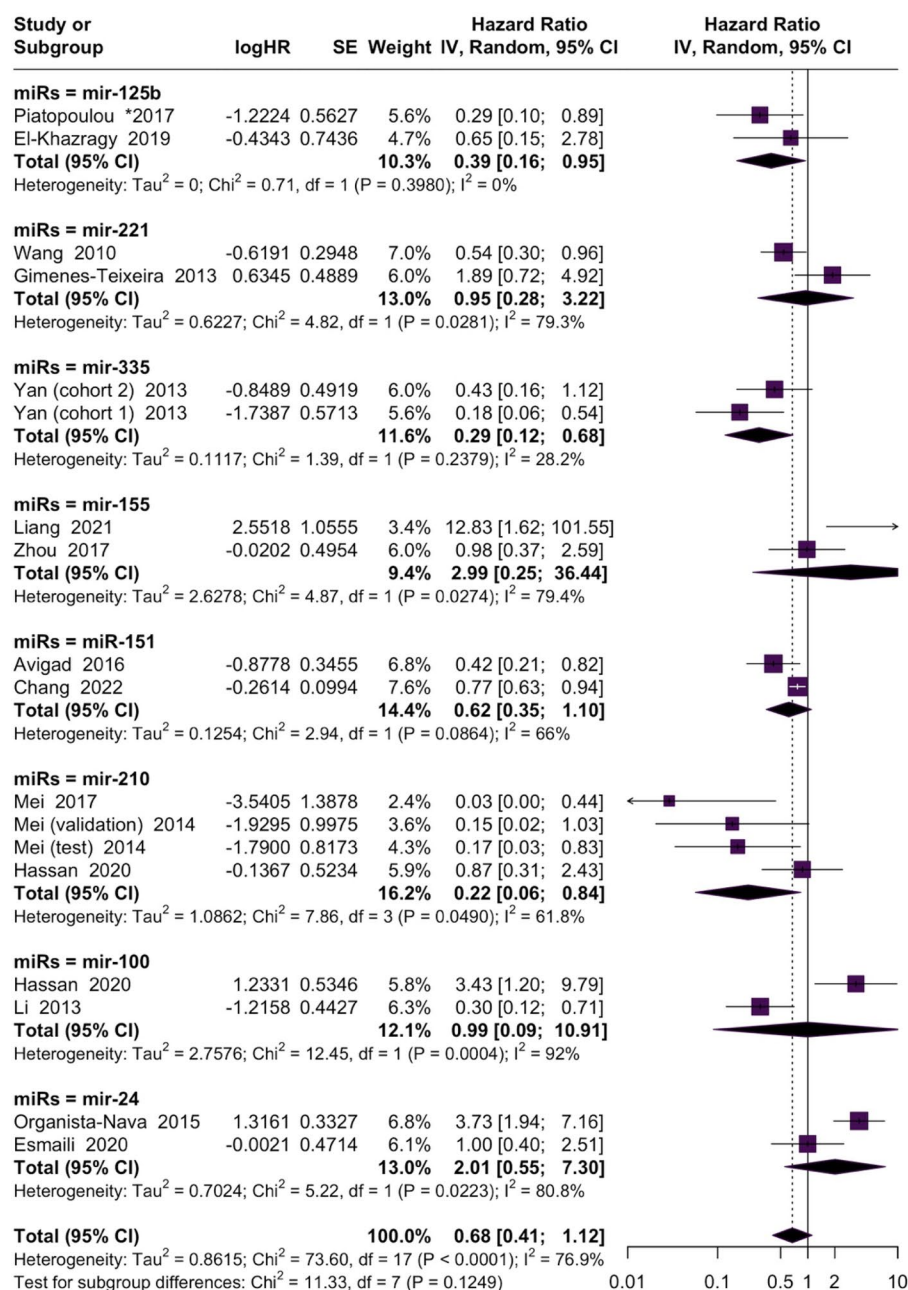


Fig. 3 The association between reported miRNAs and survival of ALL patients. miR-335, miR-210, and miR-125b were associated with improved survival in ALL patients

miRNAs into two groups based on their HR values: those with $HR > 1$ (indicating potential risk factors) and those with $HR < 1$ (indicating potential protective factors). Meta-analyses were conducted separately for each group when enough data (≥ 2 studies) were available. For PFS, where only one study was available, no meta-analysis was performed. The results for each outcome are summarized below. For OS, the miRNA risk factor miRNAs

had a pooled HR of 3.01 (95% CI, 2.07–4.38; $I^2 = 37\%$; $k = 7$), with significant heterogeneity. Protective miRNAs showed a pooled HR of 0.40 (95% CI, 0.31–0.51; $I^2 = 29\%$; $k = 13$), indicating a significant association with improved OS and moderate heterogeneity (Fig. 4a). For DFS, the pooled HR for risk-associated miRNAs ($HR > 1$) was 2.41 (95% CI, 1.19–4.91; $I^2 = 76\%$; $k = 4$), while protective miRNAs ($HR < 1$) had a pooled HR of 0.39 (95%

Table 2 Discordant prognostic effects of miRNAs across cohorts

miRNAs	Protective cohort (HR)	Risk cohort (HR)	Context likely explaining discordance
miR-100	Li 2013 (China, pediatric ALL, BM, RT-qPCR; OS/DFS): HR 0.30 (0.12–0.71)	Hassan 2020 (Egypt, pediatric ALL, BM, RT-qPCR; OS/DFS): HR 3.43 (1.20–9.79)	Same age group and specimen, but different regions; likely unmeasured clinical and treatment differences or population effects
miR-221	Wang 2010 (China, adult mixed-ALL, BM; OS): HR 0.54 (0.30–0.96)	Gimenes-Teixeira 2013 (Brazil, T-ALL, mixed pediatric/adult; OS/DFS): HR 1.89 (0.72–4.92)	Subtype + age mix differ: mixed-ALL adults vs T-ALL (and mixed ages)
miR-210	Mei 2017/Mei 2014 cohorts (China, pediatric ALL, BM; RFS/OS/DFS/EFS): HRs 0.03–0.17 (protective)	Hassan 2020 (Egypt, pediatric ALL): HR 0.87 (0.31–2.43; null/risk-leaning)	Region/cohort differences, possible treatment or biology variation; mix of reported vs KM-derived HRs
miR-125b	Piatopoulou 2017 (Greece, pediatric ALL, BM; OS/DFS): HR 0.29 (0.10–0.89)	El-Khazragy 2019 (Egypt, pediatric ALL, PB; OS/PFS): HR 0.65 (0.15–2.78; null)	Specimen + endpoint differ: BM vs peripheral blood, and OS/DFS vs OS/PFS; also different regions
miR-155	Zhou 2017 (China, adult B-ALL, BM; OS): HR 0.98 (0.37–2.59; null)	Liang 2021 (Turkey/Netherlands, pediatric ALL, BM; EFS): HR 12.83 (1.62–101.55)	Age + endpoint + subtype differences: pediatric EFS (mixed-ALL) vs adult OS (B-ALL)

CI, 0.21–0.73; $I^2 = 61\%$; $k = 6$), showing a consistent protective association (Fig. 4b). For EFS, the pooled HR for risk-associated miRNAs was 3.35 (95% CI, 0.42–26.89; $I^2 = 76\%$; $k = 2$), while protective miRNAs had a pooled HR of 0.39 (95% CI, 0.20–0.78; $I^2 = 68\%$; $k = 6$) (Fig. 4d). For RFS, risk-related miRNAs had a pooled HR of 2.73 (95% CI, 1.37–5.42; $I^2 = 36\%$; $k = 2$), while protective miRNAs showed a pooled HR of 0.30 (95% CI, 0.12–0.79; $I^2 = 54\%$; $k = 4$), indicating significant protective effects (Fig. 4c). Since only one study reported PFS, providing an HR of 0.46 (95% CI: 0.12–1.75) for miR-125b, a meta-analysis for this outcome was not performed.

Heterogeneity and sensitivity analyses (multilevel random-effects models)

Using crossed random effects for study and miRNA, variance partitioning demonstrated that heterogeneity between miRNAs was minimal across all outcomes (miRNA-level $I^2 \approx 0\%$), while dispersion was primarily at the study level. For protective miRNAs, study-level I^2 was 0% for OS but was significant for DFS (76.4%), EFS (75.0%), and RFS (63.3%). For risk miRNAs, study-level I^2 was 75.5% (OS), 73.5% (DFS), and 61.8% (EFS) (Supplementary File 4).

Consistent with this, effects were stable in qPCR-only (protective OS HR=0.43) and BM-only (protective OS HR=0.40) subsets, and heterogeneity attenuated in BM-only analyses for DFS (HR=0.29; $I^2 = 36\%$) and EFS (HR=0.22; $I^2 = 0\%$ [$k = 2$]). The pediatric-only OS subset showed larger, consistent effects (HR=3.69; $I^2 = 0\%$ [$k = 3$]). Heatmaps and restriction forests (Supplementary Figs. 2–5; Supplementary File 4) highlight specimen source and HR derivation (reported vs. KM-extracted) as principal contributors to study-level variance. Prediction

intervals (e.g., protective DFS 0.20–0.77, EFS 0.21–0.99) further reflect this between-study dispersion. Sensitivity analyses restricted to high-quality studies preserved the direction of effect (e.g., protective OS HR=0.38), suggesting that study quality alone does not explain the observed associations.

For risk-associated miRNAs, leave-one-out analyses (Supplementary Fig. 6) showed that pooled OS estimates remained stable (HR range 2.82–3.64), with heterogeneity mainly driven by [36] (I^2 dropped to 0% when omitted). Risk-DFS estimates were consistent in direction but more sensitive to individual cohorts ($k = 4$), with [45] contributing most to heterogeneity.

Publication bias

For protective miRNAs (OS), the funnel plot was approximately symmetric (Supplementary Fig. 1). Egger's test $p = 0.11$ indicated no clear small-study effects. However, for all other endpoints and risk-miRNA analyses, study counts were less than 10, so funnel and Egger assessments were not performed due to low reliability.

Target genes identification of prognostic miRNAs

For all miRNAs linked to ALL prognosis in the literature review, target genes for both human and non-human genes were identified. However, since only three miRNAs were significant in the meta-analysis, we focused on them for further analysis. Using the miRTarBase database, we identified a total of 299 validated target genes for these miRNAs, including 238 human and 61 non-human targets. Specifically, miR-125b targeted 154 genes (113 human and 41 non-human), miR-210 targeted 97 genes (82 human and 15 non-human), and miR-335 targeted 48 genes (43 human and 5 non-human). The list

of experimentally validated miRNA target genes that overlap with the differential expression gene results can be found in Supplementary File 5. Further, these target genes were cross-referenced with the DEGs from the scRNA-seq dataset.

Differential expression of miRNA target genes in scRNA-Seq data of ALL

To assess miRNA regulatory effects, we analyzed differential expression of experimentally validated miRNA target genes in human ALL single-cell datasets (Fig. 5a–f and Supplementary File 6–8). We also explored non-human miRNAs–target interactions to evaluate cross-species conservation of regulatory effects (Supplementary File 9).

B-ALL vs. normal cells

In B-cells, five genes (*HIF1A*, *MCL1*, *SOX4*, *BCL2*, and *UBQLN1*) were notably upregulated. In blast cells, 14 genes (such as *SOX4*, *ZEB2*, *DUSP6*, *HIF1A*, and *BTK*)

were significantly upregulated, while five genes (such as *STAT3* and *BNIP3*) were downregulated. In DC, *TGFBI* was significantly upregulated. In erythrocytes, four genes (*HIF1A*, *SOX4*, *TP53INP1*, *MX1*) were upregulated, while 11 genes (such as *BIRC5*, *HMGA1*, *GATA1*) were significantly downregulated. In monocytes, eight genes (such as *SOX4*, *TNFAIP3*, *STMN1*) showed increased expression, whereas three (*XIST*, *MEGF9*, *IL6R*) were downregulated. In NK cells, *TNFAIP3* and *MX1* genes were upregulated, while *XIST* was downregulated. Also, in T-cells, *TNFAIP3* and *SOX4* genes were upregulated, while *RPS6KA1* gene was downregulated (Supplementary File 6).

T-ALL vs. normal cells

In B-cells, 15 genes (such as *TNFAIP3*, *MCL1*, *HIF1A*, *BCL2*, *KRAS*) were significantly upregulated, while five genes (such as *BTK*, *RPS6KA1*, *MID1IP1*) were downregulated. In blast cells, 29 genes (such as *SOX4*, *STMN1*, *LDHA*) were upregulated, with two genes

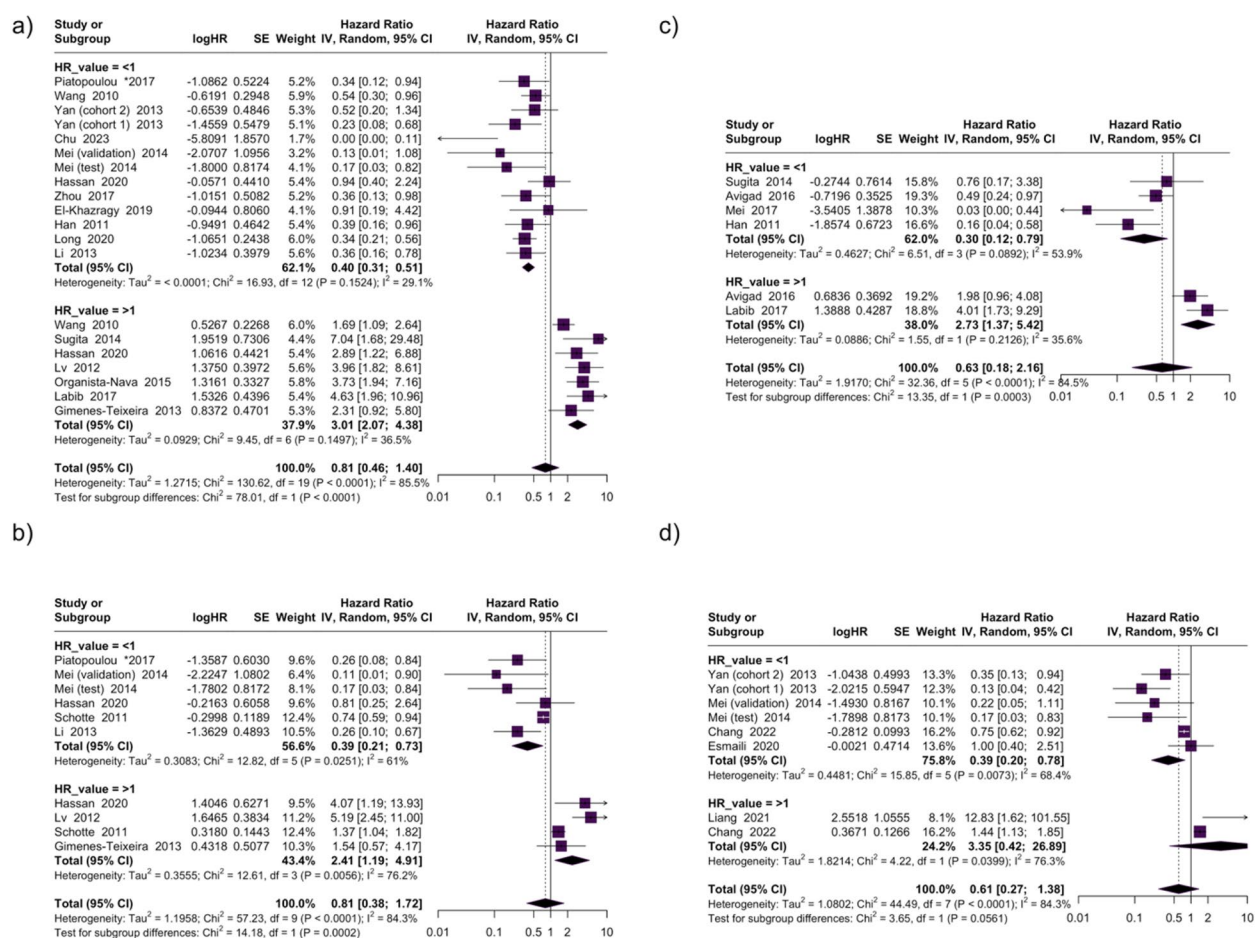


Fig. 4 Forest plots illustrating the association between protective and risk factor miRNA expression and different types of survival in ALL patients. The association between protective and risk factor miRNA expression with OS (a), DFS (b), RFS (c), and EFS (d) has been shown

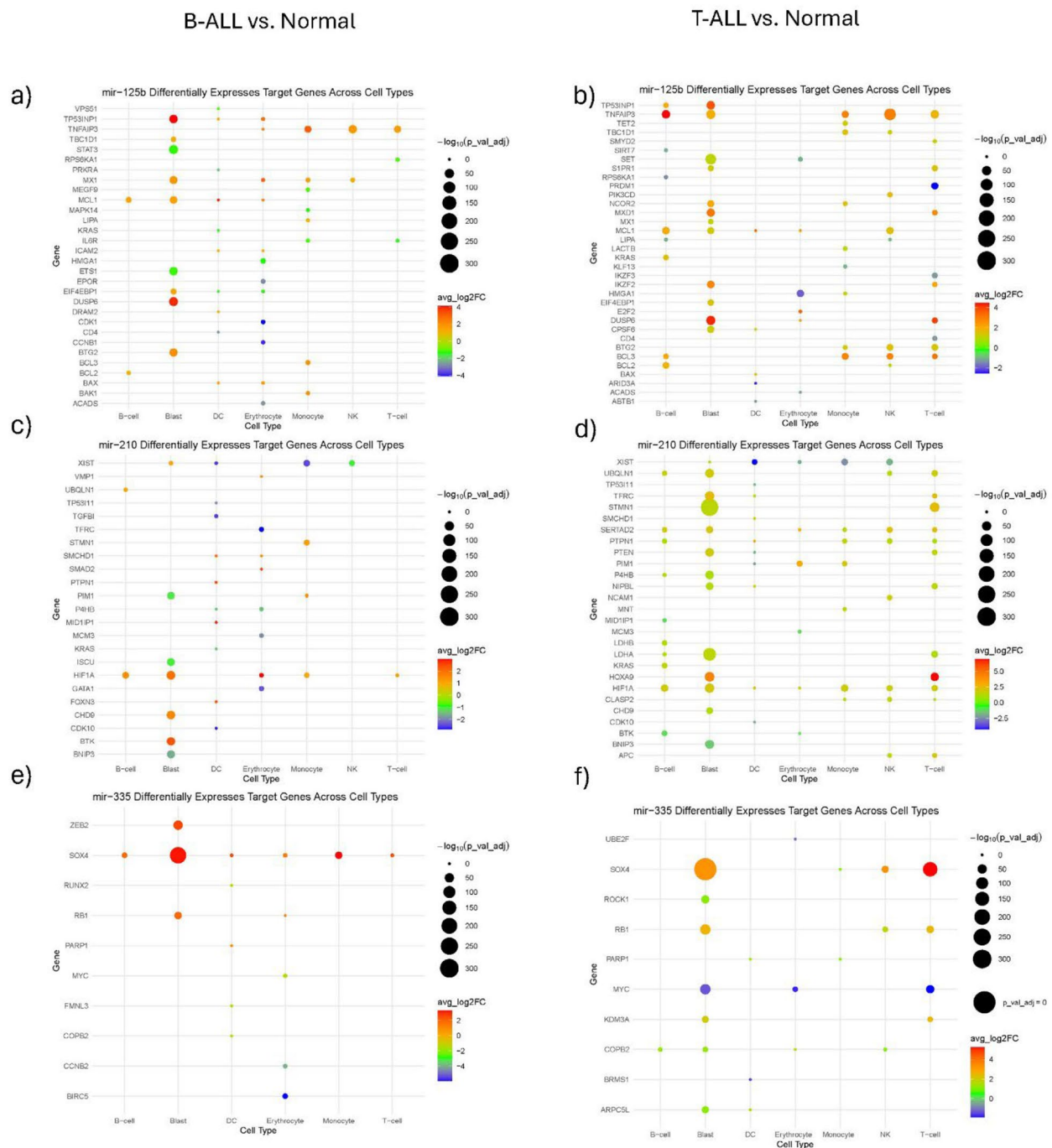


Fig. 5 Differential expression of miRNA target genes in single-cell RNA-Seq (scRNA-Seq) Data of acute lymphoblastic leukemia (ALL). Differential expression of target genes for (a, b) mir-125b, (c, d) mir-210, and (e, f) mir-335 in B-ALL and T-ALL versus normal samples, respectively

(MYC, BNIP3) downregulated. In DC, the XIST gene was significantly downregulated. In erythrocytes, the PIM1 gene was significantly upregulated, while four genes (HMGA1, MYC, SET, XIST) were downregulated. In monocytes, 14 genes (such as HIF1A, TNFAIP3, BCL3, TBC1D1) were upregulated, whereas

two genes (XIST, KLF13) were downregulated. In NK cells, 17 genes (such as TNFAIP3, MCL1, SOX4, BCL3, HIF1A) were upregulated, with XIST showing reduced expression. In T-cells, 22 genes (such as SOX4, STMN1, TNFAIP3, HOXA9) were upregulated, whereas four

genes (*MYC*, *PRDM1*, *IKZF3*, *CD4*) were downregulated (Supplementary File 7).

T-ALL vs. B-ALL cells

When comparing T-cell ALL to B-cell ALL, seven genes (such as *LDHB*, *TNFAIP3*, and *P4HB*) were significantly upregulated. In blast, 15 genes (such as *HOXA9*, *TNFAIP3*, *UBQLN1*, *PIM1*) were upregulated, while three (*BTK*, *ZEB2*, *BTG2*) were downregulated. In DC, the *SOX4* gene was significantly downregulated. In erythrocytes, 10 genes (such as *PIM1*, *TFRC*, *STMN1*, *GATA1*) were upregulated, while five (*SOX4*, *TP53INP1*, *SET*, *XIST*, *HIF1A*) were downregulated. In monocytes, six genes (such as *TET2*, *CD4*, *TBC1D1*, *LACTB*) showed increased expression, with two (*SOX4*, *MX1*) downregulated. In NK cells, 12 genes (such as *TNFAIP3*, *SOX4*, *APC*, *BCL3*) were upregulated, and *LIPA* was downregulated. In T-cells, 20 genes (such as *SOX4*, *STMN1*, *PPP1CA*, *HOXA9*, *RBI*) were upregulated, whereas *MYC* showed decreased expression (Supplementary File 8).

Overall, human single-cell analyses revealed that miR-125b, miR-210, and miR-335 target genes exhibit distinct expression patterns across all subtypes and immune cell lineages.

Discussion

This systematic review and meta-analysis evaluate the prognostic significance of miRNAs in ALL. For this purpose, OS, DFS, EFS, RFS, and PFS were evaluated in separate meta-analyses, and miRNAs were classified as protective (HR < 1) or risk-associated (HR > 1). The combined analysis revealed a notable association of protective miRNAs with improved survival for the initial four survival outcomes. In addition, miRNAs that were reported in more than one cohort were analyzed separately for their impact on ALL survival. To further explore the significant miRNAs' biological implications, the target genes of these miRNAs were identified by miRTarBase to deduce potential regulatory mechanisms. Notably, three miRNAs—miR-335, miR-210, and miR-125b—emerged as protective biomarkers.

In our meta-analysis, miR-335 was associated with a protective relationship with survival in ALL (pooled HR, 0.29 [0.12; 0.68]) in two cohorts of the same study. Interestingly, the two cohorts of the Yan study differed in the association of miR-335 with survival. Cohort 1 demonstrated a weaker protective effect, while Cohort 2 demonstrated a stronger association with more favorable survival outcomes [37]. This discrepancy is perhaps explained by differences in patient characteristics or treatment regimens, illustrating the context-dependent nature of miRNAs' prognostic roles. Furthermore, miR-335 dysregulation has been implicated in prognosis in

several other types of cancer, including gastric cancer [55, 56], non-small cell lung cancer [57], colorectal cancer [58], ovarian cancer [59], esophageal squamous cell carcinoma [60], hepatocellular carcinoma [61], melanoma [62], with low expression of miR-335 typically correlating with more advanced stages of the disease, metastasis, and poorer survival outcomes [63]. At the cellular level, miR-335 controls basic biological processes of cell cycle regulation, apoptosis, and cell invasion by targeting key oncogenic genes like *SOX4*, *BCL-w*, *ROCK1*, and *ID4* [63]. These pathways have been validated in specific cancers. For instance, in ovarian cancer, miR-335 suppresses invasion and triggers apoptosis by targeting *BCL-w* and breaking down F-actin-rich structures [64, 65]. In multiple myeloma, it promotes apoptosis as it downregulates *SOX4*, affecting downstream *AKT*, *PI3K*, and *HIF1- α* signaling pathways. Similarly, in melanoma and non-small cell lung cancer, miR-335 regulates cell cycle progression by inhibiting *Cyclin D* [66], *CDC2/CDK1*, and *CDC25*, causing G0/G1 arrest and causing limit in proliferation [62, 63, 67, 68]. However, in hematologic malignancies, the roles of miR-335 seem to be highly context-dependent. In pediatric T-ALL, miR-335-3p functions as a tumor suppressor, where its upregulation, mediated by knockdown of *CDKN2B-AS1*, inhibits leukemic cell proliferation, promotes apoptosis, and enhances sensitivity to adriamycin via downregulation of *TRAF5*. This suggests a potential therapeutic role for miR-335-3p in T-ALL by overcoming chemoresistance [69]. Furthermore, it is shown that the overexpression of exogenous miR-335 in ALL cell lines (697, RS4:11, and Sup-B15) significantly enhances sensitivity to prednisolone. Besides, as a novel regulator of *MAPK1*, miR-335 overexpression leads to reduced *MAPK1* mRNA levels in ALL cell lines. These findings suggest that downregulated miR-335 may contribute to glucocorticoid resistance by inhibiting *MAPK1* [37]. Although the role of miR-335 in AML has shown conflicting results [70–72], studies in ALL have consistently pointed to its protective role, which is consistent with our results.

Furthermore, miR-210 demonstrated a significant protective association with survival in ALL (pooled HR, 0.22 [0.06; 0.84]), suggesting its potential as a favorable prognostic biomarker. However, while most of the reported cohorts report a protective role of miR-210 [41, 43], one study suggests poorer survival outcomes in the high-expression group [44]. These findings may result from various mixtures of patient characteristics in each cohort and warrant further research. Similarly, contrasting evidence exists about the role of miR-210's in other malignancies. A former meta-analysis has indicated miR-210 as a risk-associated factor in several malignancies, including breast cancer, primary head and neck

squamous cell carcinoma, renal cancer, soft-tissue sarcoma, pediatric osteosarcoma, bladder cancer, and glioblastoma [73]. In fact, miR-210, recognized as a “hypoxia miRNA,” is mainly known as an oncogene that promotes angiogenesis, proliferation, invasion, and chemoresistance via inhibiting apoptosis and modulation of critical pathways [74–76]. Nevertheless, miR-210 can also act through tumor-suppressive pathways by targeting specific genes, including FGFR1, HOXA1, HOXA9, ISCU, MNT, E2F3, CASP8AP2, and RAD52, depending on the cellular context [77]. This dual role also appears in leukemias. For instance, in AML, increased expression of miR-210 is correlated with increased BCL-2 expression, leading to disruption in apoptosis and poor prognosis [78]. Conversely, *in vitro* studies using hematologic cell models, such as K562 cells, have shown different findings: miR-210 facilitates erythroid differentiation via GATA-1-mediated regulation of SMAD2 in hypoxic conditions and through PLC β 1 signaling pathways [79]. These findings highlight the context-dependent variability of miR-210 function in different cancer types.

MiR-125b was another microRNA correlated with better prognosis in ALL patients based on the results of two cohorts (pooled HR: 0.39 [0.16; 0.95]). However, the included studies provide contrasting results regarding the role of miR-125b as a prognostic biomarker. Piatopoulou et al. found that low miR-125b expression in childhood ALL was associated with poor response to BFM chemotherapy, as it was correlated with shorter DFS and OS [16]. In contrast, the study by El-Khazragy et al. did not show a significant correlation between miR-125b levels and patient survival outcomes [80]. These findings may be attributed to differences in sample size, methodology, or the patient cohorts studied. For example, Piatopoulou's study focused on children with precursor B-ALL. It employed multivariate regression to assess the independent prognostic value of miR-125b, confirming its potential in risk stratification and predicting poor response to therapy. In addition, Piatopoulou et al. found that miR-125b levels were lower at diagnosis than day 33 of BFM treatment, emphasizing miR-125b's role as a reliable marker of treatment resistance. Similar to our included studies, additional papers have highlighted the role of miR-125b in chemotherapy resistance across various cancers. For instance, Zhou et al. discovered that the overexpression of miR-125b led to daunorubicin resistance in leukemia cell lines such as K562, THP-1, and Jurkat by reducing apoptosis. Importantly, inhibiting miR-125b enhanced DNR cytotoxicity in REH cells, emphasizing miR-125b's role in mediating drug resistance through its regulation of apoptosis-related proteins, including G protein-coupled receptor kinase 2 and p53-upregulated modulator of apoptosis [81]. In fact, the function of

miR-125b is highly context-dependent, as it can act as a powerful oncogene in some cancers while functioning as a tumor suppressor in others [82]. This dual role is biologically justified by the different molecular targets present in various cell types. Therefore, the effect of the miRNA depends on whether it mainly silences oncogenes or tumor suppressor genes [83]. Despite the differences in outcomes, the collective evidence underscores the need to further explore miR-125b as a prognostic marker in ALL. Similar to other cancer types, its regulation of apoptosis by targeting Bcl-2 may explain its varying impacts on patient survival and chemotherapy resistance [80]. More multicenter studies are needed to clarify the inconsistencies and precisely define the role of miR-125b in ALL prognosis.

Low heterogeneity and stable directionality across sensitivity analyses highlight the reproducibility and biological plausibility of protective miRNAs as genuine prognostic markers in ALL. In the multilevel framework (crossed random effects for study and miRNA), associations are consistently aligned in direction, with most variability stemming from differences between studies rather than between miRNAs. Protective markers correlate with improved survival and remain stable when restricted to qPCR assays and bone marrow specimens. Moreover, focusing on bone marrow also reduces variability in DFS and EFS, indicating that specimen source is a key factor. Analytical choices are also important. Covariate-adjusted HRs tend to be more variable than those derived from KM estimates, possibly due to different adjustment covariates in various analyses. Similarly, age seems to have an influence, with pediatric groups displaying more consistent patterns than mixed or adult groups. Therefore, based on heterogeneity heatmaps and restriction forests, specimen source and HR calculation methods are the main sources of variability, though the direction of effects remains consistent across groups [84, 85]. To evaluate potential publication bias, we analyzed funnel plot symmetry and conducted Egger's regression test, where the number of studies allowed. For the protective OS, the funnel plot appeared roughly symmetric, and Egger's test did not show clear small-study effects ($p=0.11$). For the other outcomes and risk-miRNA analyses, the number of studies was less than 10, so funnel plots and Egger's tests were not performed due to limited reliability. However, the small number of studies for several endpoints means that small-study effects or selective reporting cannot be definitively ruled out. Prospective registration of prognostic studies and standardized reporting of miRNA cut-offs and survival endpoints in large, multicenter cohorts will be important for more accurately assessing publication bias and confirming the reproducibility of these prognostic signals.

To understand the survival biomarkers (miR-335, miR-210, miR-125b) in a mechanistic context, we integrated human, experimentally validated miRNA–target interactions from miRTarBase with scRNA-seq data. These initial analyses demonstrate cell-type-specific enrichment of apoptosis and survival pathways (BCL2, MCL1) [86–88], hypoxia/stress responses (HIF1A) [89], and developmental transcription factors along with cytoskeletal regulators (SOX4, ROCK1, RB1, MYC) [90–93], which align with previous findings. The targets are consistently upregulated in blasts, B-cells, and T-cells of ALL, supporting the idea that miR-335 and miR-210 may have a protective role, as higher miRNA levels would suppress these pro-survival and proliferative pathways. Notably, leukemic blasts showed the most consistent and significant changes in miRNA target expression, indicating they are the main cell population responsible for miRNA-related dysregulation in ALL. These single-cell results offer a mechanistic explanation for the observed survival advantage, connecting protective miRNAs to the suppression of anti-apoptotic and hypoxia-response pathways within blasts. We also performed an orthologue-aware exploratory analysis of targets validated in non-human species, mapped to human one-to-one orthologues. Although gene-level overlap is limited—as expected due to 3' UTR divergence—the pathway-level signals are consistent: non-human targets emphasize mitochondrial metabolism and stress response (UCP2, ND6, RHEB) [94–96], MAPK/AP-1 signaling (JUN, ERK3/MAPK6) [97, 98], and RNA processing (PRPF8, KIF13B) [99, 100]. These align with the human-target modules previously discussed (hypoxia, apoptosis, proliferation). Notably, some nodes (BCL2, HIF1A) are shared across both levels, highlighting the evolutionary conservation of the apoptosis–hypoxia axis in ALL biology. In contrast, human-exclusive signals (e.g., ROCK1, SOX4) highlight processes related to adhesion, cytoskeletal dynamics, and lineage specification, with clearer relevance to hematopoiesis. Human-validated targets paired with scRNA-seq expression provide supportive, correlative evidence of dysregulated programs in specific immune-cell compartments; they help prioritize manageable hypotheses for mechanistic testing (e.g., miR-335: SOX4/ROCK1, miR-210: HIF1A/BCL2, miR-125b: BCL2/IRF4/ARID3A). Conversely, the non-human layer is hypothesis-generating. It broadens the mechanistic space (mitochondrial and MAPK nodes) but is not used for therapeutic inference without experimental validation in human ALL. Overall, these analyses suggest that the main, conserved axis linking our protective miRNAs to outcomes is repression of anti-apoptotic and hypoxic

survival circuits, while context-dependent differences (such as SOX4/ROCK1 prominence in T-ALL) probably reflect lineage-specific regulatory wiring [101, 102].

Strengths and limitations

Our study extends previous reviews of miRNAs in ALL [20, 24, 25] by enhancing both methodology (a comprehensive search from 2010 to 2023 to include both adult and pediatric ALL (T-ALL and B-ALL), addressing the underpowered aspect in earlier meta-analyses) and biological interpretation. First, using multilevel random-effects models with crossed random effects for study and miRNA, we identified where heterogeneity exists—localizing heterogeneity to the study level (with negligible between-miRNA variance) and producing PIs that reflect between-study uncertainty for clinical interpretation. Second, by analyzing protective and risk miRNAs separately and conducting preplanned restriction analyses (bone-marrow-only, RT-qPCR-only, pediatric-only, reported vs. KM-extracted HRs, region, and study quality). Third, by reconstructing HRs from KM curves, we extended the quantitative scope compared to previous reviews—thereby increasing the evidence base and stabilizing pooled estimates by avoiding the exclusion of studies that only reported KM curves. Finally, integrating experimentally validated targets with single-cell RNA-seq revealed cell-type-specific dysregulation, offering mechanistic hypotheses that link prognostic signals to leukemic biology. These advancements provide clinically meaningful, direction-specific estimates and a prioritized list of miRNA candidates for future validation. Our results also emphasize the importance of standardization (BM-based, RT-qPCR assays) to reduce heterogeneity and accelerate translation.

However, most cohorts of our study came from Asia, the Middle East, or Europe and mainly included pediatric populations. Adult-only datasets had limited power. As a result, validation for other populations and adult ALL still needs to be confirmed. Moreover, less than half of the studies were of moderate quality, with inconsistent reporting of missing data and follow-up. Although survival assessment is generally objective, the lack of blinding and variable covariate adjustment may bias individual HRs. However, restricting to high-quality studies preserved the effect direction but not all heterogeneity. Furthermore, heterogeneous dichotomization (median vs. ROC-derived vs. quantiles) hinders cross-study comparability and may lead to overly optimistic effects in data-driven analyses thresholds. Therefore, standardized, pre-defined cut-offs should be prioritized.

Conclusion and perspectives

From a translational perspective, miRNAs have promising assay qualities—short length, stability outside cells, detectability in plasma or serum, and suitability for multiplex qPCR panels—while the clinical pipeline now includes various diagnostic and prognostic tests, along with renewed therapeutic efforts using safer oligonucleotide chemistries and tumor-targeted delivery systems.

Through a multilevel meta-analysis encompassing 24 cohorts, miR-335, miR-210, and miR-125b emerge as protective in ALL. Single-cell analyses of experimentally validated targets offer a mechanistic explanation for their associations with survival. These findings require thorough validation before clinical implementation. For instance, luciferase reporter assays and loss- or gain-of-function experiments in ALL models are essential to establish causation, not just correlation.

For clinical translation, we suggest a stepwise plan: (1) standardize pre-analytical and analytical procedures (specimen type, RNA handling, qPCR panels); (2) set fixed thresholds based on training sets and confirm in independent, multi-center cohorts; (3) define predetermined endpoints (OS/DFS/RFS/EFS) with consistent covariate adjustments; and (4) assess additional benefits over current risk tools (MRD, cytogenetics) using c-index, net reclassification, calibration, and decision-curve analyses. Completing these steps and validating the findings through large, prospective, multi-center trials will determine whether these miRNAs should be incorporated into future risk stratification strategies in ALL. Future studies should also assess whether miR-335, miR-210, and miR-125b offer additional prognostic value beyond established tools such as MRD status, cytogenetic and molecular risk markers (e.g., IKZF1 alterations), and high-risk subtypes including Ph-like ALL.

Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AMKL	Acute megakaryocytic leukemia
BCR-ABL1	Breakpoint Cluster Region-Abelson Murine Leukemia Viral Oncogene Homolog 1
BM	Bone marrow
BMT	Bone marrow transplantation
CAR-T	Chimeric antigen receptor T-cell
CLL	Chronic lymphocytic leukemia
CMMML	Chronic myelomonocytic leukemia
CNS	Central nervous system
COG	Children's Oncology Group
CR	Complete remission
CRLF2	Cytokine receptor-like factor 2
Cy	Cyclophosphamide
DFS	Disease-free survival
EFS	Event-free survival
FAB	French–American–British Classification
FACS	Fluorescence-activated cell sorting
FISH	Fluorescence in situ hybridization
HCVAD	Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone)

HLA-DR	Human leukocyte antigen-DR isotype
IGH	Immunoglobulin heavy chain
IL3	Interleukin 3
KMT2A	Lysine methyltransferase 2A
miRNA/miR	MicroRNA
MRD	Minimal residual disease
NPM1	Nucleophosmin 1
NOS	Newcastle-Ottawa Scale
OS	Overall survival
PB	Peripheral blood
Ph+	Philadelphia Chromosome Positive
Ph-	Philadelphia Chromosome Negative
PI3K	Phosphoinositide 3-kinase
PFS	Progression-free survival
PR	Partial remission
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RFS	Relapse-free survival
RUNX1	Runt-related transcription factor 1
SCT	Stem cell transplantation
t(9;22)	Translocation between chromosomes 9 and 22 (Philadelphia chromosome)
TCF3-PBX1	Transcription factor 3—pre-B cell leukemia homeobox 1 fusion gene
TdT	Terminal deoxynucleotidyl transferase
TKI	Tyrosine kinase inhibitor
T-ALL	T-cell acute lymphoblastic leukemia
T-ALL/LBL	T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13643-026-03083-3>.

Supplementary Material 1: Search query.
 Supplementary Material 2: Quality assessment.
 Supplementary Material 3: miRNAs for each outcome.
 Supplementary Material 4: Restriction analysis.
 Supplementary Material 5: Target genes of miRNAs.
 Supplementary Material 6: Results of sc-RNAseq: B-ALL vs. healthy.
 Supplementary Material 7: Results of sc-RNAseq: T-ALL vs. healthy.
 Supplementary Material 8: Results of sc-RNAseq: T-ALL vs. B-ALL.
 Supplementary Material 9: Results of sc-RNAseq based on non-human target genes.
 Supplementary Material 10: Funnel plot.
 Supplementary Material 11: Heterogeneity across sensitivity analysis in protective miRNAs.
 Supplementary Material 12: Heterogeneity across sensitivity analysis in risk miRNAs.
 Supplementary Material 13: Forest plot of subgroup analysis in protective miRNAs.
 Supplementary Material 14: Forest plot of subgroup analysis in risk miRNAs.
 Supplementary Material 15: Leave-one-out; DEGs based on non-human target genes.

Acknowledgements

The authors would like to thank all the staff of the Hematology, Oncology, and Stem Cell Transplantation Research Center at Tehran University of Medical Sciences, Tehran, Iran.

Authors' contributions

Conceptualization: ZS and ST; data extraction: KB, MA, MM, KI; meta-analysis: ST; miRNAtargets identification: KB, MA and MM; single-cell RNAseqanalysis:

NS and FM; writing—original draft preparation: ST, FM, and FM, and ZE; writing—review and editing: ST, NS, and ZS; illustration: ZE; project administration and supervision: ZS. All Authors approved the final version to be published. They all agreed to be accountable for all aspects of the work.

Funding

This work was supported by Tehran University of Medical Sciences (TUMS) (Grant number: 1403-3-107-74077).

Data availability

The data supporting this systematic review's findings are available from the corresponding author upon reasonable request. Readers can access the data by emailing the corresponding author.

Declarations

Ethics approval and consent to participate

This systematic review was conducted following the principles of the Declaration of Helsinki. Ethical approval for this review was obtained from the Hematology, Oncology, and Stem Cell Transplantation Research Center, Tehran. The ethical approval code for this study is IR.TUMS.HORCSC.TEC.1403.056.

Competing interests

The authors declare that they have no competing interests.

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Received: 23 June 2025 Accepted: 15 January 2026

Published online: 29 January 2026

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