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# A Meta-Analysis of microRNA profiling data in Acute Myeloid Leukemia

# Μετα-ανάλυση δεδομένων έκφρασης microRNA στην Οξεία Μυελογενή Λευχαιμία

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# I. Abstract

**Introduction:** Acute Myeloid Leukemia (AML) is a heterogenous disease characterized by the abnormal proliferation of myeloid leukemic blasts in the bone marrow. Leukemogenesis is a complex process that results from deregulation of several cellular processes. Several studies show that microRNA (miRNA) deregulation contributes to AML pathogenesis.

**Objectives:** A meta-analysis to decipher the AML miRNA deregulation compared to healthy controls and to investigate possible roles of significantly deregulated miRNAs.

**Methods:** A systematic literature database and Genome Expression Omnibus (GEO) database search was implemented with defined eligibility criteria for study inclusion. Study derived miRNA lists were prioritized and analyzed with the robust rank aggregation (RRA) method. Bonferroni correction was implemented. Significant miRNAs were further studied through target prediction analysis and pathway enrichment analysis.

**Results:** From the literature dataset 12 studies were included for analysis, as well as 7 studies from the GEO database. The RRA method implementation and Bonferroni correction resulted in a 5 miRNA (hsa-miR-9, hsa-miR-31, hsa-miR-139-5p, hsa-miR-145, hsa-miR-181a-3p) meta-signature. Subsequent, target prediction analysis resulted in multiple miRNA associated targets and enrichment analysis showed several distinct cellular processes.

**Conclusion:** A five miRNA meta-signature that reached statistical significance is found to be deregulated in AML compared to healthy controls.

**Keywords:** acute myeloid leukemia; microRNA; meta-analysis; systematic review; target prediction analysis; pathway enrichment analysis.



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# I. Περίληψη

**Εισαγωγή:** Η οξεία μυελογενής λευχαιμία (ΟΜΛ) αποτελεί μια εξαιρετικά ετερογενή νόσο που χαρακτηρίζεται από τον ανεξέλεγκτο πολλαπλασιασμό μυελικών βλαστών στον μυελό των οστών. Η λευχαιμογένεση αποτελεί μια πολύπλοκη διαδικασία που προκαλείται από την διαταραχή πολλαπλών κυτταρικών λειτουργιών. Πολλαπλές μελέτες δείχνουν ότι η απορρύθμιση πολλαπλών microRNA (miRNA) συνεισφέρει στην παθογένεση της ΟΜΛ.

**Στόχοι:** Η διενέργεια μετα-ανάλυσης για την αποσαφήνιση της απορρύθμισης των miRNA στην ΟΜΛ σε σχέση με υγιείς μάρτυρες και η μελέτη πιθανών βιολογικών λειτουργιών σημαντικά απορυθμισμένων miRNA.

**Μέθοδοι:** Διενεργήθηκε συστηματική ανασκόπηση βιβλιογραφικών και γενετικών (Gene Expression Omnibus- GEO) βάσεων δεδομένων με προκαθορισμένα κριτήρια συμπερίληψης. Οι λίστες miRNA που προέκυψαν κατηγοριοποιήθηκαν βάσει σημαντικότητας και αναλύθηκαν μέσω της μεθόδου robust rank aggregation (RRA). Στα αποτελέσματα εφαρμόσθηκε διόρθωση Bonferroni. Στατιστικά σημαντικά miRNA αναλύθηκαν ακολούθως μέσω ανάλυσης πρόβλεψης στόχου και ανάλυσης εμπλουτισμού.

**Αποτελέσματα:** Από την βιβλιογραφική ανασκόπηση προέκυψαν 12 μελέτες για περαιτέρω ανάλυση, ενώ από την GEO 7 μελέτες. Η εφαρμογή της μεθόδου RRA και της διόρθωση Bonferroni ανέδειξε 5 miRNA (hsa-miR-9, hsa-miR-31, hsa-miR-139-5p, hsa-miR-145, hsa-miR-181a-3p) με στατιστικά αλλαγή στην έκφραση μεταξύ πασχόντων και μαρτύρων. Οι επακόλουθες αναλύσεις πρόβλεψης στόχου και εμπλουτισμού ανέδειξαν πολλαπλούς mRNA στόχους και πολλές διαφορετικές εμπλεκόμενες κυτταρικές λειτουργίες αντίστοιχα.

**Συμπεράσματα:** Πέντε miRNA φαίνεται πως έχουν στατιστικά σημαντική διαφορά στην έκφραση μεταξύ των ασθενών με ΟΜΛ και υγειών μαρτύρων.

**Λέξεις-κλειδιά:** οξεία μυελογενής λευχαιμία; microRNA; μετα-ανάλυση; συστηματική ανασκόπηση; ανάλυση πρόβλεψης στόχου; ανάλυση εμπλουτισμού.



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## II. Introduction

Acute Myeloid Leukemia (AML) represents a heterogenous group of myeloid neoplasms characterized by the abnormal proliferation and infiltration of the bone marrow (BM) by poorly differentiated clonal blast cells [(1)]. AML represents the most common acute leukemia in adults, with a median age at diagnosis of 65 years [(2)(3)], whereas in children and adolescents is rare [(4)]. 5-year-survival in AML patients is low, with chemoresistance and disease relapse occurring frequently [(5)].

Regarding disease subtype classification, there exist two main classification systems which integrate molecular, cytogenetic, clinical, and morphological features: the World Health Organization (WHO) 5$^{th}$ edition of myeloid and histiocytic/dendritic neoplasms [(6)] along with the earlier published 4$^{th}$ edition [(7)] and the International Consensus Classification of myeloid neoplasms and acute leukemia [(8)]. Earlier, classifications systems (such as the widely used French- American- British classification [(9)] relied more in morphology, maturation features and clinical characteristics. The published literature analyzed herein relies primarily on the 4$^{th}$ edition of the WHO classification and the FAB classification (*Table 1*).

| FAB classification | Disease Name |
|---|---|
| M0 | Undifferentiated acute myeloblastic leukemia |
| M1 | Acute myeloblastic leukemia with minimal maturation |
| M2 | Acute myeloblastic leukemia with maturation |
| M3 | Acute promyelocytic leukemia |
| M4 | Acute myelomonocytic leukemia |
| M4Eos | Acute myelomonocytic leukemia with eosinophilia |
| M5 | Acute monocytic leukemia |
| M6 | Acute erythroid leukemia |
| M7 | Acute megakaryoblastic |

*Table 1: French- American- British (FAB) classification of AML.*

In de novo AML, gene sequencing has revealed that AML cases harbor at least 10 mutations which can be broadly categorized in nine domains of cellular functions that affect leukemogenesis: DNA methylation, transcription factor (TF) fusions, tumor suppressors, nucleophosmin function, cell signaling, chromatin modification, myeloid TFs, cohesion complex and spliceosome complex [(10)]. The most frequently mutated genes include: fms like tyrosine kinase 3(FLT3) (28 percent), nucleophosmin1 (NPM1) (27 percent), DNA-methyltransferase 3A (DNMT3A) (26 percent), among others [(10)(11)]. Cytogenetic alterations also contribute greatly to the pathogenesis of AML, with the most common being: translocation t(15;17) (q24.1;q21.2) and variants (13 percent), trisomy 8 (10 percent), t(8;21)(q22;q22.1) and variants (7 percent), 11q23.3 rearrangements (6 percent), inversion inv(16)(p13.1q22)/t(16;16)(p13.1;q22) (5 percent) [(12)].

Leukemogenesis is a multistep process resulting in the leukemic transformation of a myeloid precursor cell. The two-hit hypothesis of leukemogenesis suggests that the leukemic clone arises from at least two genetic lesions [(13)]: class I mutations which provide the clone with a



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proliferative advantage (e.g., FLT3-ITD mutations) and class II mutations which halt differentiation (e.g., CEBPA mutations) [14].

MicroRNAs (miRNAs) represent a class of small, non-coding RNAs with a length of 19~ 22 nucleotides, that play a major role in post-transcriptional gene regulation and as a result affect many cellular processes proliferation, differentiation, apoptosis and hematopoiesis [15]. Consequently, deregulated miRNA expression in AML might aide leukemic transformation through multiple pathways.

In recent years, an increasing number of studies have demonstrated that miRNAs in AML cases are differentially expressed in contrast to healthy individuals, along with an association between miRNA signature and AML subtype. However, no meta-analysis of published articles and public database data has been conducted to date.

# III. Methods

## Search strategy and study selection

A systematic database search was conducted for the identification of microRNA profiling studies in AML, in a two- level fashion. Firstly, a comprehensive literature database search was performed. From July 11th 2023 to July 29th 2023, the databases PubMed, Google Scholar, Science Direct were thoroughly researched using the terms: "Acute Myeloid Leukemia", "AML", "microRNA", "miRNA". The initial results were screened based on relevance and eligibility. After the initial screening the included studies were assessed for eligibility and relevance based on title and abstract. The included studies were assessed as full text for eligibility. All duplicates were removed. Secondly, a database search on the publicly available genomic databases "Gene Expression Omnibus (GEO)" was performed with a similar screening process. All database searches were conducted in accordance with the *PRISMA 2020 guidelines* [16] and the resulting PRISMA flowchart was created using *PRISMA2020* [17].

## Eligibility criteria

Due to the inherent high degree of heterogeneity in these kinds of studies, a strict set of eligibility criteria was implemented: 1) Human studies, 2) Case- healthy control study design, 3) No prior treatment for AML at the time of sampling, 4) No review articles, 5) English language, 6) No cell line data, 7) No Tumor Cancer Genome Atlas (TCGA) data, 8) No preselected miRNA (when the preselected miRNA number was under three).

## Data extraction

Lists of available microRNA lists were extracted, along with their expression values and significance values- if available. Furthermore, a set of data from each individual article- such as: author, publication year, number of miRNA probes, platform used, number of cases and controls- were extracted. Some studies produced lists of differentially expressed miRNAs, through comparison of disease subtype (e.g., FAB subtype or Cytogenetic lesion) with healthy controls. These lists were treated autonomously without pooling the study data into a single list.



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## Statistical analysis

All extracted data were processed using Microsoft Excel. Regarding literature database miRNA lists: individual miRNA names were standardized through the latest miRbase version and all expression data (e.g., fold change, or counts per million) were log 2 transformed (if this was not already done by the authors). MiRNA lists were then sorted and ranked based on the absolute expression value, in a descending order. This was a necessary preprocessing step before the implementation of the robust rank aggregation (RRA) method [18]. The RRA algorithm assumes that imported data are ranked based on significance (i.e., differential expression change), and the data are then compared to a null model that assumes random order of input lists. The output lists contain p- values assigned to each element (miRNA). False positive results were dealt with the Bonferroni correction method. For the GEO databases, the GEO2R [19] and GEOexplorer [20] online webtools were at first utilized to generate the lists of differentially expressed miRNAs. Then the top 200 miRNAs from each ranked miRNA list were also analyzed through the RRA algorithm. Graphs were created using IBM SPSS Statistics Data Editor version 29.0.0. Significance was set to a p- value of 0.05.

## Target prediction and Enrichment analysis

The meta- signature miRNAs were selected for target prediction analysis by using TargetScan v.8 [21] and DIANA microT- CDS 2023 webserver [22]. The top five miRNA targets from each algorithm were selected based on context score (for TargetScan) and interaction scores (for microT- CDS). Furthermore, DIANA TarBase v8 [23] was used for experimentally validated target identification. Gene targets that overlapped between the prediction algorithm results or were found to be also experimentally identified, were further analyzed through the Ensembl Database (Release 110) [24].

Pathway identification of predicted miRNA targets was carried out through DIANA miRPath v4 [25] using pathway union analysis, target selection from TarBase v8 and pathway selection from the Kyoto Encyclopedia of Genes and Genomes (KEGG), as well as Genome Ontology (GO).

When meta-signature miRNAs were not reported as mature miRNAs based on the latest nomenclature (e.g., hsa-miR-9 and not has-miR-5p or hsa-miR-3p) both mature miRNA strands were used for the target prediction and pathway enrichment analysis.

# IV. Results

## Study selection and data extraction

Database search initially resulted in 946 publications from literature databases and 185 publications from the GEO database with 12 studies and 7 studies ultimately meeting the inclusion criteria- through a rigorous process (Figure 1). Four studies [39][41][42][44] were deleted from the literature results due to overlap with the GEO database results.



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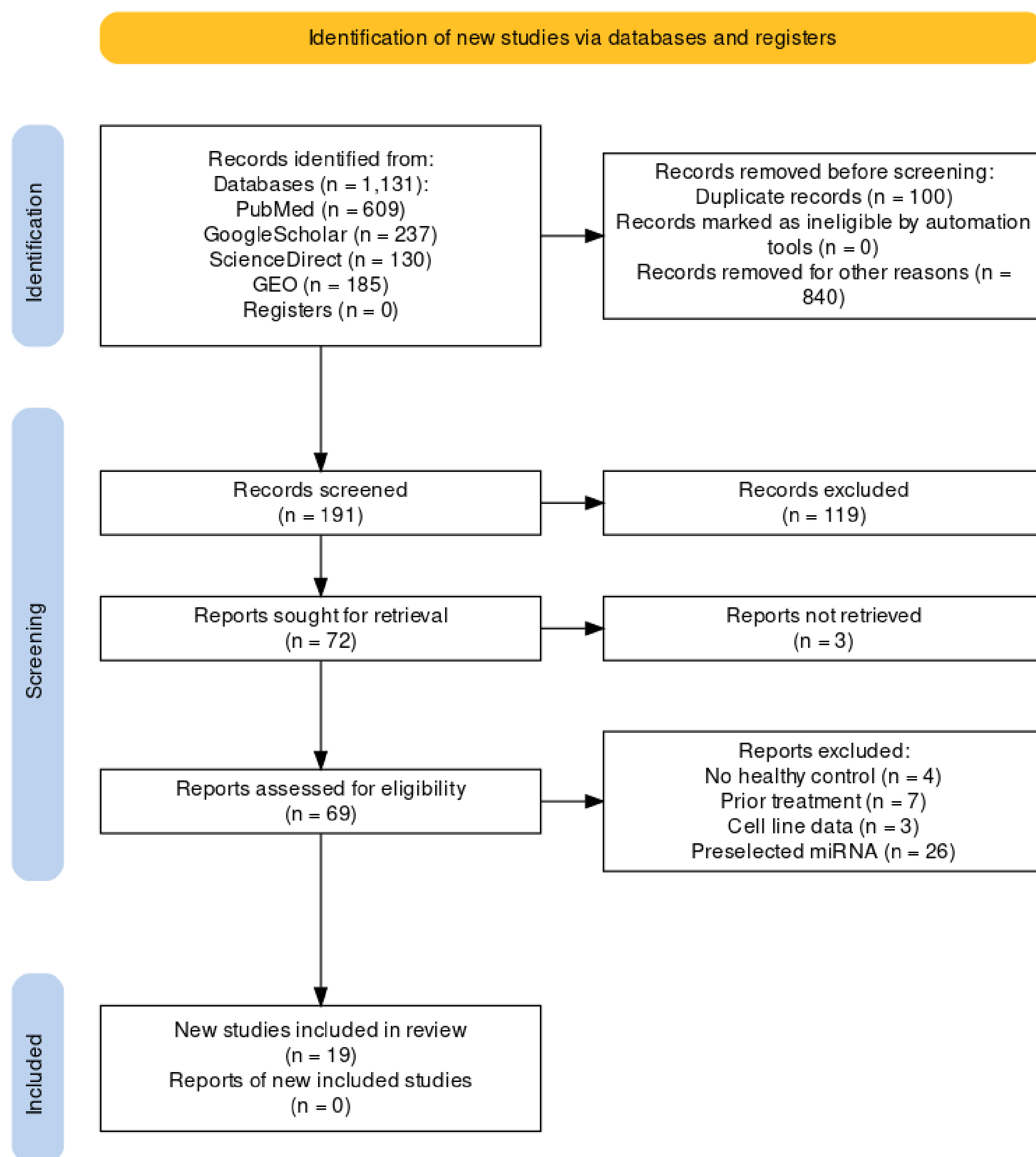


*Figure 1: The resulting PRISMA flowchart.*

Regarding the literature database results, five studies [28][29][30][35][36] were not used in the meta-signature analysis due to limited data availability. Most studies used quantitative real time polymerase chain reaction (qRT-PCR) or a microarray method for differential miRNA expression identification. Average miRNA probe number was 368, with a range between 10 and 1488 miRNA probes- depending on the platform used. A total of 942 AML patients and 272 healthy controls were included. The majority of studies used FAB- classification or (cyto)genetic lesions as patient categorization and subsequent comparative analysis. Concerning the FAB



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classification: 19 patients classified as M0, 43 as M1, 221 as M2, 121 as M3, 140 as M4, 156 as M5, 18 as M6, 7 as M7, 36 were characterized as not otherwise specified (NOS) and 71 patients were not categorized based on the FAB classification.

Apropos of the GEO database studies, most studies were conducted using either next generation sequencing or microarray. Average probe number was 792 (ranging from 559 to 851). A total of 133 AML patients and 74 controls were analyzed.

A summary of characteristics of the included studies can be found in Table 2 and Table 3.

| Author | Year | Cases | Controls | miRNA | Platform | Age |
|---|---|---|---|---|---|---|
| Fayyad-Kazan et al (26) | 2013 | 20 | 20 | 380 | TaqMan Human MicroRNA Array A | Adult |
| Cammarata et al (27) | 2010 | 29 | 3 | 365 | TaqMan Custom Array | Adult |
| Memari et al (28) | 2022 | 40 | 50 | 10 | ABI157 PRISM 7500 real-time PCR System | N/A |
| Lutherborrow et al (29) | 2011 | 27 | 8 | 1488 | miRCURY LNA microRNA probe set | Adult |
| Isken et al (30) | 2007 | 50 | 12 | 154 | TaqMan master | Adult |
| Moussa Agha et al (31) | 2020 | 31 | 11 | 380 | TaqMan Human MicroRNA Array A | Adult |
| Zhi et al (32) | 2013 | 140 | 135 | 25 | Solexa sequencing analysis | Adult |
| Jongen-Lavrencic et al (33) | 2008 | 215 | 4 | 260 | TaqMan microRNA assay | Adult |
| Garzon et al (34) | 2008 | 122 | 10 | N/A | TaqMan miRNA assays, microarray assay | Adult |
| Daschkey et al (35) | 2013 | 102 | 3 | 493 | TaqMan miRNA Assays, miRXplore Microarray | Paediatric |
| Ding et al (36) | 2018 | 156 | 10 | N/A | N/A | Adult |
| Wang et al (37) | 2012 | 10 | 6 | 123 | miRCURY LNA arrays | Adult |

*Table 2: Literature dataset studies.*



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| Author | Year | ID | Cases | Controls | Platform | Age |
|---|---|---|---|---|---|---|
| Vanhooren J et al [38] | 2022 | GSE196886 | 11 | 7 | Illumina NextSeq500 | Paediatric |
| Wieser R et al [39] | 2013 | GSE49665 | 52 | 13 | miRCURY LNA arrays | Adult |
| Esa E et al [40] | 2019 | GSE142699 | 24 | 24 | NanoString nCounter Human miRNA | Adult |
| Middleton F et al [41] | 2019 | GSE128079 | 10 | 9 | Illumina NextSeq500 | Adult |
| Abdelhammed S et al [42] | 2021 | GSE159028 | 12 | 12 | Illumina NextSeq500 | Adult |
| Link DC et al [43] | 2010 | GSE24222 | 1 | 1 | Agilent-021827 Human miRNA Microarray | Adult |
| Leoncini PP et al [44] | 2022 | GSE209871 | 23 | 8 | nCounter Human v2 miRNA Expression Assay | Paediatric |

*Table 3: GEO dataset studies.*

## Meta- Analysis

A meta-analysis for significantly differential expressed miRNAs was conducted for each database (literature- derived studies and GEO- derived studies). From the literature database 24 miRNAs were found to be differentially expressed with an unadjusted p- value < 0.05. However, after Bonferroni correction only 4 miRNAs were found to be significantly deregulated (hsa-miR-9, hsa-miR-31, hsa-miR-139-5p, hsa-miR-145). The meta-signature of the GEO database revealed a four- miRNA signature that reached statistical significance with an unadjusted p- value. Not surprisingly, after the Bonferroni correction only one miRNA (hsa-miR-181a-3p) was of marginal statistical insignificance with an adjusted p- value of 0.055, nevertheless it was included for further analysis. Accumulatively, the resulting meta- analysis revealed a five- miRNA signature, that was further analyzed.

The most significantly deregulated miRNA was hsa-miR-9. In the literature databases it was reported in 3 out of 12 studies [31][32][33]. Jongen-Lavrencic et al [33] report a significant downregulation of hsa-miR-9 (with the exception of the 11q23 rearranged cohort that was upregulated). Zhi et al [32] also report significant downregulation of hsa-miR-9. In contrast, Moussa-Agha et al [31] report an increase in miR-9 levels. Regarding the GEO dataset, hsa-miR-



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9 was found to be downregulated in two studies (GSE159028 [42] and GSE209871 [44]) without being statistically significant. Another differentially expressed miRNA of high significance (hsa-miR-31) presents a more uniform downregulated expression pattern among studies. In the literature dataset it is reported as significantly decreased in expression in three studies [27][33][37], as well as being reported as downregulated in the GEO study GSE142699 [40] albeit with a p- value > 0.05. The remaining significant miRNAs (hsa-miR-5p, has-miR-145) and hsa-miR-181a-3p present with inconclusive reports regarding their regulation trend.

On a genome level, the deregulated meta-signature resides in different scattered genomic loci, apart from hsa-miR-9 and hsa-miR-181a-3p that are located in chromosome 1. The deregulated meta-signature p- values and chromosomal locations are found in *Table 4*.

| microRNA | p- value | Adjusted p- value | Chromosome |
|---|---|---|---|
| hsa-miR-9 | 0.000002105326 | 0.001814791 | 1q22 |
| hsa-miR-31 | 0.00001162547 | 0.010021155 | 9p21.3 |
| hsa-miR-139-5p | 0.00001162547 | 0.010021155 | 11q13.4 |
| hsa-miR-145 | 0.00004713528 | 0.040630611 | 5q32 |
| hsa-miR-181a-3p | 0.00003944961 | 0.0553478 | 1q32 |

*Table 4: The five miRNA meta- signature of AML.*

## Target prediction and enrichment analysis

The resulting meta- signature of miRNAs that reached statistical significance (along with hsa-miR-181a-3p) were further analyzed for target prediction and pathway enrichment analysis. A summary of target counts for each miRNA in each database can be found in *Figure 2*. MiR-9 (both hsa-miR-9-3p and hsa-miR-9-5p) has the most targets in all database searches, whereas the hsa-miR-181a-3p has the least. The top five gene targets for each individual miRNA predicted in TargetScan and DIANA microT-CDS, as well as the number of experimentally identified interactions through TarBase search, can be found in *Table 5*. Top gene targets that overlapped either between the prediction algorithm results or TarBase results were further analyzed with Ensembl Release 110. Four out of six protein coding genes recorded are transcription factors (*T- box transcription factor 1* (TBX1, ENSG00000184058), *POU class 2* homeobox 1 (POU2F1, ENSG00000143190), *one cut homeobox 2* (ONECUT2, ENSG00000119547), *Y-box binding protein 3* (YBX3, ENSG00000060138)) associated with blood/ lymphatic vessel development (TBX1), transcription regulation (POU2F1), organ morphogenesis/ cell fate and migration (ONECUT2) and GM-CSF promoter regulation (YBX3). Besides these, another gene target is *fascin actin- bundling protein 1* (FSCN1, ENSG00000075618) associated with actin filament assembly, cytoskeleton organization and cell motility. The sole experimental target of hsa-miR-181a-3p is *mitochondrial ribosomal protein S35* (MRPS35- ENSG00000061794) that is a functional component of mitochondrial translation.



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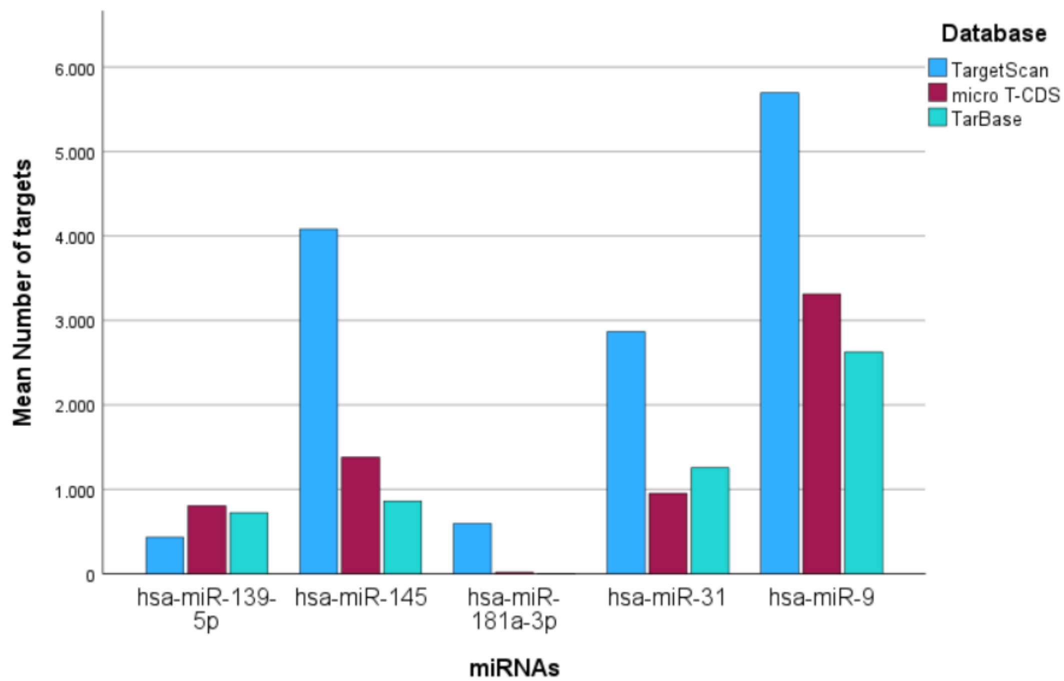


*Figure 2: miRNA target count per miRNA and database.*

| microRNA | Database | Targets |
|---|---|---|
| hsa-miR-9 | TargetScan (-3p) | *ZNF99, ACTL6A, DSCC1, MZT1, NAA20* |
| | TargetScan (-5p) | *ONECUT2, YBX3, LYVE1, POU2F1, ONECUT1* |
| | microT-CDS | *ONECUT2, POU2F1, RIVF111, YBX3, MAGT1* |
| | TarBase | *2628 Interactions* |
| hsa-miR-31 | TargetScan (-3p) | *POLR2K, ZMYM5, HAUS4, PCP4, RHOXF4* |
| | TargetScan (-5p) | *RNF144B, RSBN1, SH2D1A, AK4, PAX9* |
| | microT-CDS | *RSBN1, SSX2B, KRT36, VAT1L, MTAP* |
| | TarBase | *1255 Interactions* |
| hsa-miR-139-5p | TargetScan | *TBX1, H2AZ2, DPY30, ARL15, MORN4* |
| | microT-CDS | *PDE3A, CDH20, DCC, TBX1, PRDM16* |
| | TarBase | *724 Interactions (including TBX1)* |
| hsa-miR-145 | TargetScan (-3p) | *DST, SLC30A5, SPINK13, SCT, COA1* |
| | TargetScan (-5p) | *CSRNP2, FSCN1, ABHD17C, MYO5A, FLI1* |
| | microT-CDS | *NMNAT3, SRGAP2, DAB2, FSCN1, TRIM2, FLI1* |
| | TarBase | *861 Interactions (including FSCN1)* |
| hsa-miR181a-3p | TargetScan | *NAP1L5, MAP3K5, ARL4A, AGPAT4, DEXI* |
| | microT-CDS | *RIBC1, TAL1, DDX41, TRAPPC4, DIMT1* |
| | TarBase | *1 Interaction (MRPS35)* |

*Table 5: Top miRNA target genes per database.*



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Through miRPath v.4 pathway enrichment analysis was performed. Several pathways from GO and KEGG pathways were found to be significant. In the GO analysis, the most frequent associated pathways were protein binding related and nucleoplasm – cytoplasm compartment associated. In the KEGG analysis, cellular signaling pathways and cancer associated pathways were the most enriched. The top enriched pathways from GO and KEGG are reported in *Table 6*.

| Term Name | Term Genes | miRNAs (n) | Merged FDR |
|---|---|---|---|
| **GO** | | | |
| protein binding | 14297 | 7 | 4,4016E-137 |
| nucleus | 7177 | 7 | 1,8673E-113 |
| cytosol | 5614 | 6 | 8,00253E-92 |
| cytoplasm | 7391 | 5 | 2,93076E-89 |
| nucleoplasm | 4107 | 6 | 7,48813E-87 |
| **KEGG** | | | |
| Neurotrophin signaling pathway | 124 | 3 | 1,37569E-08 |
| MAPK signaling pathway | 329 | 3 | 1,01191E-07 |
| Focal adhesion | 213 | 2 | 1,01191E-07 |
| Transcriptional misregulation in cancer | 206 | 3 | 2,09337E-07 |
| Salmonella infection | 277 | 2 | 3,19031E-07 |

*Table 6: Top GO compartments and KEGG pathways enriched by the meta- signature miRNA targets.*

# V. Conclusion

Through robust rank aggregation, 7 and 21 prioritized miRNA lists that were extracted from the GEO and Literature datasets respectively, ultimately identified a set of 4 significant deregulated miRNAs (hsa-miR-9, hsa-miR-31, hsa-miR-145, has-miR-139-5p) between AML patients and healthy controls from the literature dataset and one miRNA that was marginally not statistically significant from the GEO dataset (hsa-miR-181a-3p).

The trend of deregulation of each miRNA is not concise across reports of individual studies, apart from hsa-miR-31 being downregulated athwart different studies. The most significantly deregulated miRNA, hsa-miR-9, was found to have not a uniform trend of deregulation across studies. Jongen-Lavrencic et al [(33)] and Zhi et al [(32)] showed that hsa-miR-9 is downregulated compared to normal controls. However, in the subset of 11q23 rearranged (mixed linage leukemia-(MLL-)) AML in the study by Jongen-Lavrencic et al [(33)], hsa-miR-9 was significantly upregulated, a finding that is confirmed by Chen P et al [(45)]. Moreover, abnormally increased hsa-miR-9 expression might be a negative prognostic factor in AML [(46)]. Important predicted targets include transcription factors like ONECUT1 and 2, POU2F1 and YBX3 among others



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(*Table 5*), signifying the pleiotropic effects of this miRNA in cellular function. The second most deregulated miRNA that showed consistent downregulation in AML patients across studies is hsa-miR-31. Interestingly, hsa-miR-31-5p is found to be repressed in human leukemic stem cells (LSCs), with its restoration eliminating LSCs and halting AML progression [(47)]. Regarding, hsa-miR-139-5p and hsa-miR-145 their expression levels in AML patients varied among different studies in both the GEO and Literature datasets. As far as has-miR-181a-3p is concerned, even though its deregulation resulted in an adjusted p- value of 0.055, it was included in further analyses. The deregulation signature is also found to be inconclusive between the included studies. However, its downregulation is associated with adverse outcome in cytogenetically normal (CN-) AML [(47)].

AML is a highly heterogenous malignancy, evident by the different classifications and the diverse prognosis. Several of the included studies, compared different AML subtypes (either by FAB type, cytogenetic or molecular lesion) with healthy controls, that showed specific miRNA signatures can be associated with AML subtypes [(35)(34)(33)]. However, in the present study a subgroup meta- analysis of AML subtype specific meta- signature was not conducted.

The present study contains several limitations due to data availability, data preprocessing and the inherent heterogeneity of included studies due to different platforms utilized and different data processing methods. Nevertheless, the resulting miRNA meta-signature provides interesting starting points for further basic and clinical AML research.

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