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MicroRNAs and tRNA-derived fragments predict the transformation of myelodysplastic syndromes to acute myeloid leukemia

Yan Guo¹, Stephen A. Strickland², Sanjay Mohan², Shaoying Li³, Amma Bosompem⁴, Kasey C. Vickers⁵, Shilin Zhao⁶, Quanhu Sheng⁶, and Annette S. Kim⁷

¹Center for Quantitative Sciences, Vanderbilt University, Nashville, TN

²Department of Medicine, Division of Hematology/Oncology, Vanderbilt University Medical Center Nashville, TN

³Hematopathology Department, The University of Texas MD Anderson Cancer Center, Houston, TX

⁴Department of Pathology, Vanderbilt University Medical Center Nashville, TN

⁵Department of Medicine, Vanderbilt University Medical Center Nashville, TN

⁶Department of Cancer Biology Vanderbilt University, Nashville, TN

⁷Department of Pathology, Brigham and Women's Hospital, Boston, MA

Abstract

Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders of the elderly that carry an increased risk of progression to acute myeloid leukemia (AML). Since small non-coding RNAs (sRNAs), including microRNA (miRNAs), act as regulators of cellular differentiation, we hypothesized that changes to sRNAs might be implicated in the progression of MDS to AML. We conducted sRNA sequencing on three sets of patients: Group A (MDS patients who never progressed to AML); Group B (MDS patients who later progressed to an AML); and Group C (AML patients with myelodysplasia-related changes, including patients with a known preceding diagnosis of MDS). We identified five miRNAs that differentiated Groups A and B, independent of bone marrow blast percentage, including three members of the miR-181 family, as well as differential patterns of miRNA isoforms (isomiRs) and tDRs. Thus, we have identified sRNA biomarkers that predict MDS cases that are likely to progress to AML.

Keywords

Myeloid Leukemias and Dysplasias; Prognostication; Molecular genetics

Corresponding Author: Annette S. Kim, Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115, Tel: (617) 525-3172, Fax: (617) 264-5169, askim@bwh.harvard.edu.

POTENTIAL CONFLICTS OF INTEREST

There are no other conflicts of interest. The authors have each completed an ICMJE Disclosure Form.

INTRODUCTION

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell disorders with defects in effective hematopoiesis, resulting in cytopenias clinically and dysplasia pathologically. MDS occurs most commonly in the elderly with a median age of 70, with an incidence of up to 75/100,000.¹ While survival in patients with only unilineage dysplasia and a good prognostic score may not differ significantly from the background normal population, while those with excess blasts have a median survival of as little as nine months. In patients who succumb to the disease, the most common cause of death is related to cytopenias, such as infection and bleeding; however, a subset of MDS patients will transform to an acute myeloid leukemia (AML), the rate of transformation ranging from less than 2% to up to 33% in the subtype of refractory anemia with excess blasts-2 (RAEB-2).² Those patients who transform to AML have extremely poor prognosis.

Despite the updated prognostic systems,³⁻⁵ it is still unclear at the time of diagnosis of MDS which patients will ultimately transform to AML. Routine laboratory testing has poor predictive value in estimating the likelihood of progression to AML in patients without excess blasts. Although parallel sequencing methods have identified progression-related mutations acquired at the time of transformation to AML,⁵⁻⁹ it would be ideal to identify those patients likely to progress well before they accumulate increased blasts to allow for potential intervention, such as allogeneic stem cell transplantation. Therefore, a goal of this research has been to identify aberrations that predict *future* transformation to AML.

There are fundamental biologic differences between MDS and AML. MDS is characterized by ineffective maturation while AML is characterized by a block in differentiation. As regulators of cell differentiation, microRNAs (miRNAs) play proven roles in the development of MDS. In 5q minus syndrome, a subtype of MDS, miR-145 and miR-146 are harbored in the common deleted region of 5q, and their knockdown appears to be associated with the observed thrombocytosis and dysmegakaryopoiesis.¹⁰ Our group and others have published miRNA signatures of non-5q minus subtypes of MDS.¹¹⁻¹⁶ In addition, miRNAs that characterize AML have been identified.¹⁷⁻²² We hypothesize that miRNAs are also implicated in the development of the maturation block that characterizes MDS transformation to AML.

miRNAs are single-stranded small non-coding RNAs (sRNAs) of 19-25 nucleotides in length, which repress translation by binding to the 3' untranslated regions of the messenger RNAs (mRNAs) through complementary seed sequences, situated at positions 2-8 in 5' terminal end of the mature miRNA.²³⁻²⁵ Through translational repression, miRNAs tightly regulate cellular identity and differentiation. Conventionally annotated miRNAs in miRBase are considered canonical miRNAs.²⁶ In addition, there are alternative isoforms of miRNAs (isomiRs) which are altered at either 5' or 3' terminal ends of the mature sequence and potentially regulate different target mRNAs than the parent miRNA.

It has recently become apparent that another type of sRNA also appears to regulate translation in a miRNA-like manner, transfer RNA (tRNA)-derived sRNAs or fragments (tDRs or tRFs). Although these tDRs were discovered through RNA sequencing methods

and initially discarded as artifact, there is increasing literature pointing to these small oligonucleotides as *bona fide* biological entities with roles in translational regulation as well as in viral infection, oxidative stress, and cancer.²⁷⁻³³ The mature tRNA is produced from the actions of RNaseP and RNaseZ on the precursor tRNA, cleaving the 5' and 3' trailer sequences, respectively. The tRF-1 series is derived from the latter 3' trailer sequence. The actions of various other endonucleases are implicated in the biogenesis of the tRF-5 or tRF-3 series (cleaving from the 5' and 3' ends, respectively), including the miRNA-processing enzyme, Dicer, amongst others.²⁹ The tRF-5s typically are ~15, ~22, or ~32 nts in length (tRF-5a, -5b, and -5c, respectively, the 5cs also known as tRNA halves (tRHs)), while common tRF-3s are ~18 and ~22 nts in length (tRF-3a and -3b). In addition, intermediate tRF species have also been identified without a terminal 5' or 3' end from the mature tRNA, such as the V-type series.³²

High-throughput sequencing permits the simultaneous interrogation of all these different species of sRNAs that are known to regulate cellular differentiation and phenotype. With the goal of identifying sRNAs that predict those MDS patients likely to transform early to AML, we conducted RNA sequencing on formalin-fixed paraffin embedded particle preparations of bone marrow aspirate samples from 19 MDS patients with no known progression to AML (Group A), 11 samples from patients with MDS that later transformed to AML (Group B), and 10 samples from patients with AML, with either a known or presumed prior MDS (Group C) (Figure 1).

MATERIALS AND METHODS

Patient samples

All patient samples were obtained with approval from the Institutional Review Board at Vanderbilt University Medical Center. Samples were identified by querying the electronic laboratory information system (LIS) at Vanderbilt University Medical Center (Triple G®) for archival bone marrow biopsy studies using the terms “myelodysplastic syndrome,” “refractory cytopenia,” or “refractory anemia” in the diagnostic lines from 2004 through 2011, excluding cases of non-5q minus MDS. Samples were selected for RNA isolation based upon adequacy of the formalin-fixed paraffin-embedded (FFPE) particle preparations. Ultimately, FFPE samples were collected from 19 MDS specimens with no known progression to AML within 2 years of follow-up (Group A), 11 samples from patients with MDS that later transformed to AML within 2 years (Group B), and 10 samples from patients with AML, either secondary to a known prior MDS or with a presumed prior history of MDS (i.e. multilineage dysplasia at the time of the diagnosis of AML and history of preceding cytopenias) (Group C). Associated laboratory and clinical data were obtained from the medical records (Table 1). All categorizations of MDS were made originally using the 2008 World Health Organization (WHO) classification since the study preceded the 2016 WHO revisions.^{2,34} However, the typical conversions are noted in Table 1.

RNA Extraction

FFPE particle preparations were extracted using the Recoverall Total RNA Extraction from FFPE™ kit (Applied Biosystems, Foster City, CA). Isolated RNA was quantified using the Qubit RNA assay and quality controlled on Agilent Bioanalyzer.

Small RNA Sequencing

Library construction and sequencing was performed as described previously.^{35,36} In short, library construction from total RNA used the TruSeq Small RNA sample preparation kit (Illumina, San Diego, CA) and sequencing was performed on the Illumina HiSeq2500. Raw sequencing reads in BCL format were processed through CASAVA-1.8.2 for FASTQ conversion and demultiplexing. The RTA chastity filter was applied and only PF (pass filter) reads were retained for further analysis.

Sequencing Data Analysis

We implemented a custom sRNA sequencing data analysis pipeline.³⁶ After trimming 3' adapters using Cutadapt,³⁷ quality control was performed on the raw data using QC3.³⁸ All reads with lengths less than 16 nucleotides were removed. The adaptor-trimmed reads were formatted into a non-redundant FASTQ file containing the read sequence and copy number for each unique tag. The usable unique reads were mapped to the whole (human) genome using Bowtie1³⁹ with only one mismatch allowed. Our pipeline takes into consideration non-templated modifications⁴⁰⁻⁴³ at the 3' end of mature miRNAs during alignment, resulting in more accurate miRNA expression quantification. The tRNA and other small RNA databases were prepared by combining the latest UCSC tRNA database GtRNAdb⁴⁴ with the tRNA loci of mitochondria from the Ensembl database.⁴⁵ The reads mapped to tRNA loci were used not only for tRNA quantification, but also for tRNA mapping position coverage analysis. Non-coding sRNAs were divided into three major categories: miRNAs, tDRs, and other sRNAs (including sRNAs-derived from parent long non-coding RNAs, snoRNAs, snRNAs, and other miscellaneous RNAs in the Ensembl database). miRNA isomiRs were detected by matching alignment of the reads at +1 and +2 positions from the start of 5' annotation.

Expression Data Analysis

Expression data quality control was performed using MultiRankSeq.⁴⁶ We only considered sRNAs to be reliably detected if the standard deviation of read count was greater than 10 across all 40 samples. This filter ensured that we sequenced sufficient depth for RNA expression analysis, and only the reliably detected miRNAs were used in further analyses. Differential expression analysis was carried out using DESeq2.⁴⁷ The Benjamini Hochberg⁴⁸ adjusted p-value < 0.05 was considered to be significantly differentially expressed.

RESULTS

Patient Characteristics

Archival bone marrow samples (FFPE particle preparations) were collected from 19 MDS specimens from 16 patients with no known progression to AML within 2 years of follow-up (Group A), 11 samples from 7 patients with MDS that later transformed to AML within 2 years (Group B), and 10 samples from 9 patients with AML, with either a known or presumed prior MDS (Group C). The clinical and laboratory characteristics of these patients are summarized in Table 1. In Group A, 84% of samples had less than 5% blasts in the bone marrow. In Group B, only 45% of samples had less than 5% blasts in the bone marrow. In Group C, 44% of patients had a preceding diagnosis of MDS (i.e. a secondary AML), while the remaining 5 patients had a presumed diagnosis of a preceding MDS. The average length of follow-up for all patients in Group A was 45 months (68 months for surviving patients), 19 months for Group B (all deceased), and 23 months for Group C (57 months for surviving patients).

Differential Expression of sRNAs Between Groups A, B, and C

We identified 244 miRNAs, 46 miRNA isomiRs, 321 tDRs, and 558 other sRNAs (Table S1-S4) with sufficient reads in the samples for downstream analysis. For the comparison Group B vs A, we identified 9 differentially expressed miRNAs; for the comparison of Groups C vs A, we identified 13 differentially expressed miRNAs; for the comparison of Groups C vs B, we identified one differentially expressed miRNA (Table 2). These findings suggest that the biggest difference in expression is between Group A versus either B or C. In particular, our analysis identified a significant increase in the expression of the miR-181 family in patients in Groups B or C, as compared to patients in Group A. Of note, 18 of the 23 differentially expressed miRNAs listed above have been previously identified in related AML studies.^{14,18-20,49-58} Out of these 18 miRNAs, 15 showed identical directionality of the expression change (i.e. increased or decreased expression) when comparing AML to normal samples.

Differentially Expressed isomiRs

We also performed differential expression analysis of miRNA isomiRs. For the comparison of Groups B vs A, we identified 2 differentially expressed isomiRs, 25 differentially expressed isomiRs between Groups C and A, and 5 differentially expressed isomiRs between Groups C and B (Table 3). Interestingly, the majority of the canonical miRNA forms for the differentially expressed isomiRs were not themselves differentially expressed. Only the miRNAs in miR-181 families were differentially expressed in both canonical and isomiR forms. Taking miR-181b-5p and its isomiR miR-181-5p+1 as an example (Figure 1A), we demonstrated that the resultant differences in the seed regions caused a substantial difference in the repertoire of predicted targets (Figure 1B, manuscript in preparation).

miRNAs that Can Distinguish Groups A and B Independent of Blasts and Cytogenetics

Since there is a substantial difference in the blast percentage by group (Figure 2A), the ability of the miRNA-181 family to distinguish Groups B and C from Group A might be due

to the increased proportion of cases in Group B that had increased blasts. Indeed, the miRNA-181 family has been implicated previously both in the discrimination of cases of AML from normal controls^{14,18,49} and from high risk MDS from low risk disease, a distinction partly made on the bases of blast count.^{15,16} We therefore interrogated the miRNA expression patterns using linear regression with blast percentage as the outcome and miRNA expression level as the primary predictor. Adjusting for age, we did not find any miRNAs associated with blast percentage after adjusting for multiple comparisons using the Benjamini Hochberg false discovery rate approach. Furthermore, we conducted logistic regression using Groups A and B as the outcome and the differentially expressed miRNAs between A and B as the primary predictor after adjusting for blast percentage and age. We found five miRNAs with predictive power between Groups A and B after adjusting for multiple comparisons (Table 4). Four of these miRNAs are increased in Group B (miR-486-5p, miR-181a-5p, miR-181b-5p, and miR-181d-5p) while miR-199b-5p expression was decreased in Group B compared to Group A. Out of these five miRNAs, three were from the miR-181 family (Figure 2B-D). The same analyses were conducted for the isomiRs, but we did not find any isomiRs that could distinguish between Groups A and B after adjusting for blast percentage and age.

This finding was further refined when Groups A and B were broken down by 2008 WHO categories, although the numbers are very small and comparisons could not be made across all categories (Table S5). There were no significant differences between the miRNAs expressed by the Group A cases of refractory cytopenia with multilineage dysplasia (RCMD) (n=14, 74%) and RCMD cases in Group B (n=4, 36%). However, several miRNAs were able to distinguish the cases of RAEB (combining cases of RAEB-1 and RAEB-2) in Group A (n=3, 16%) from Group B (n=6, 55%). While many of these miRNAs may have reached significance due to the small case numbers, it is notable that again miR-181a-5p and miR-181b-5p were identified as over-expressed in Group B, further emphasizing the prognostic significance of these miRNAs independent of blast count.

Groups A and B were further delineated by cytogenetics, using the International Prognostic Scoring System criteria for determining poor risk cytogenetics.³ Within Group A, 7 cases fell into the poor risk cytogenetics category (37%) compared to 3 cases in Group B (27%). There were no significant discriminatory miRNAs between these two subgroups. Similarly, there were no discriminatory miRNAs between the poor risk and non-poor risk cytogenetic cases within Group A. Only within Group B were several miRNAs identified that were differentially expressed between poor risk and non-poor risk cytogenetic subgroups (Table S6), and none of these miRNAs were correlated with future progression to AML.

Pathway Analysis of Putative miRNA Target that Predict Transformation to AML

Using mirPath analysis tools, we identified key pathways predicted to be regulated by the 5 miRNAs listed in Table 4.⁵⁹ Frequently targeted pathways involve stimulation by various growth factors, signaling through AKT/mTOR and MAPK pathways, with multifocal effects on cell cycling and developmental transcriptional programming (Figure 3, Table S7). Based up TargetScan, these signatures are driven predominantly by increased expression of the miR-181 family, predicted to translationally repress multiple members in the AKT/mTOR

pathways including ITGA3, ITGB8, PIK3R3, AKT3, PTEN, and PPP2R5E. Synergistically, decreased expression of miR-199b-5p is also predicted to up-regulate ITGA3, GSK3B, CDKN1B, and PPARGC1A in the AKT/mTOR pathway. In addition, overexpression of the miR-181 family may repress SOS1, MAP2K1, MAPK1, CALM1, and ETS1 while miR-199b-5p is predicted to regulate SOS2 and ETS1 in the MAPK pathway.

Differential Expression of tDR

In comparing B vs A, we also identified 37 tDRs that are differentially expressed, 67 tDRs that are differentially expressed between Groups C and A, and 97 tDRs that are differentially expressed between Groups C and B (Table S8). The tDR alignment pattern analysis (5' end on the left, 3' end on the right) showed clear differences in tDR species and cleavage patterns between the groups (Figure 4). There are two discrete populations of tDRs, one of 33 nucleotide 5' tDRs that encompasses the D loop with terminal cleavage before the anticodon.³¹ The second group is composed of residues 46-72, encompassing the variable loop and the T loop of the full tRNA, similar to the type V set of tRFs that have been previously identified,³² and may represent truncated 3' tRHs. In general, only certain tRNA families (coding for the same amino acid) are represented in each group. Since each tRNA family may be composed of several isotypes (anticodons) and individual sequences (different tRNA sequences for each anticodon), this appears to be non-random differential cleavage, as has been shown previously.^{27,29,31,32} Group C had significantly more tDRs for Val (adjusted $p = 0.01$) and Gly (adjusted $p = 0.017$). In addition, there was a proportionately higher fraction of reads aligned to positions 46-72 in Groups A and B than in Group C, with greater representation of phenylalanine-encoding tDRs in Group B (adjusted $p = 9.3E-05$ for C versus B). More detailed statistical analysis using DESeq2 identified five types of anticodon tRNAs between Group B and A, four types of anticodon tRNAs between Group C and A, and seven types of anticodon tRNAs between Group C and B that were significantly differentially expressed (Table 5). The decreased expression of the tDR-Asp family in Group B as compared to Group A is the most significant change in that comparison ($p = 0.001$) and may help identify cases of MDS that are likely to progress to an AML.

DISCUSSION

This study identifies numerous sRNA species that are differentially expressed in MDS and AML, specifically those that may help predict those patients who are likely to transform to an AML within the subsequent two years. The study has focused primarily on miRNAs, their isomiRs, and tDRs, although other sRNA species were examined as well (supplementary materials, Table S9). This study was challenging due to the need for particle preparations from which adequate RNA could be obtained since non-decalcified materials are required and there was a dearth of fresh frozen samples. This led to the inclusion of cases with increased blasts in Group B, requiring additional analysis to adjust for this. In addition, paired pre- and post-transformation samples were rare, so the majority of the cases in Group C were obtained from cases of AML with either a known or presumed preceding diagnosis of MDS. Lastly, the heterogeneity of the cell populations in these particle preparations creates an added level of complexity to any interpretation of the data. Although the cases were carefully selected, it is impossible to ensure that similar proportions of the

three lineages (and their component maturational stages) and other bone marrow elements are equivalently represented. Since the particle preparations also contain full ranges of maturation of the lineages, these results are not directly comparable to other worthy studies that examined specifically CD34+ cells.^{11,16,18}

We found five miRNAs that differentiate MDS patients that progress to AML from those that do not, including four over-expressed miRNAs in patients who later progressed to AML (miR-181a-5p, miR-181d-5p, miR-181b-5p, and miR-199b-5p) and one under-expressed (miR-465-5p). Strikingly, three of these are from a single miRNA family. Several studies have identified the miR-181 members as regulators of myeloid differentiation and AML. In fact, over-expression in particular of miR-181a was observed in both AML patients^{14,18,49,60} compared to normal controls and the entire family in the high risk MDS patients compared to low risk MDS patients.¹⁵ Since the classification of high risk MDS is partially dependent upon the percentage of blasts and since AML by definition has greater than 20% blasts, the expression of miR-181 family members might correlate simply with increased blasts. Here, we have shown that the expression of these miRNAs are independent of blast count, and therefore marks other underlying biology that may be associated with progression to AML. Indeed, miR-181a-5p and miR-181b-5p are initially up-regulated in the maturation from CD34+ CD38- hematopoietic stem cells to CD34+ CD38+ hematopoietic progenitor cells, then strongly down-regulated during further maturation along granulocytic or monocytic lineages.¹⁸ In combination with our results, these findings suggest that over-expression of these miRNAs may impair maturation, thereby explaining the association of their over-expression in cases that acquire a block in maturation and transformation to AML. In contrast, in *de novo* cases of AML, this miRNA has been shown to have a tumor suppressor-like function and an association with better prognosis cytogenetics or CEBPA mutated status.⁶⁰ Similarly, the fourth over-expressed miRNA, miR-199b, has also been previously suggested to possess tumor suppressor-like functions.⁶¹ By contrast, little is known about the role of miR-486-5p in hematopoiesis.

Examination of the predicted targets of these five miRNAs identifies key signaling through the AKT and MAPK pathways (Figure 3). In particular, upregulation of the miR-181 family should have inhibitory effects on multiple members of the AKT/mTOR pathway, including PTEN,^{62,63} noted to have decreased expression in cells from high-risk MDS patients.⁶⁴ Since miR-199b-5p also regulates many inhibitors of the pathway, the targets may synergize to affect AKT signaling in MDS cells which are likely to progress to AML.

We have previously identified differentially expressed tDRs in MDS, specifically in the prediction of patients likely to respond to therapy with DNA methyltransferase inhibition.³⁵ In this study, we again identified intriguing patterns of tDR expression that correlate with whether or not a patient will progress to AML (Figure 4). The study produced several striking outcomes. First, the biased distribution of tRNA families to either the 5' tRHs series (positions 1-33) or the modified V-type series and/or 3' tRHs (positions 46-72) points to non-random production of tDRs. Secondly, the different proportion of tDR sub-species in AML compared to MDS support a biological phenomenon rather than random cleavage events potentially due to technical artifact in sequencing. Finally, the differential pattern of tRNA families within the tDRs in Groups A, B, and C allow for discrimination of these

groups on the bases of their tDR expression. It should be noted that many of the tRNA isotypes not only have the same or similar anticodons, but also have highly homologous sequences 5' or 3' to the anticodon. Therefore, although the parent tRNAs may be distinct, the corresponding tDRs may be degenerate. Hence, we have only denoted them by their general tRNA family (full sequence information available in the supplementary material). In addition to a wide range of other biological functions, tDRs also inhibit the cap-binding function of eIF4F and bind to YB-1, both of which result in translational repression.^{29,30,65} It is therefore not surprising that dysregulation of tDR expression has also been implicated in cancer.³⁰ This study and our prior study³⁵ are the first to demonstrate the utility of tDRs as biomarkers that inform the prognosis of MDS, both in the prediction of response to therapy and progression to AML.

Lastly, we examined the expression patterns of isomiRs in MDS. IsomiRs are alternative forms of miRNAs that are shifted at the 5' or 3' end by a small number of nucleotides. From the 5' end, imprecise cleavage by Drosha or Dicer can cause the matured form of miRNA to miss the first one or two nucleotides. Alternatively, post-transcriptional modifications can add or remove nucleotides to the 3' end of miRNA, resulting in non-templated additions. Previous studies have found that 5' isomiRs are rarer than 3' isomiRs.^{66,67} However, because of 5' variation produces alternative seed sequences compared to the canonical miRNA form, 5' isomiRs might exhibit alternative biological properties distinct from their canonical form. We now show that isomiRs are also differentially expressed in MDS that does or does not progress to AML.

In summary, recent literature has identified roles of tDRs and isomiRs in translational regulation and therefore cellular differentiation, roles that were once thought to be exclusive to miRNAs. With the growing maturity of high-throughput sequencing technology, researchers can now interrogate all these sRNA species through a single platform of miRNA-seq. Using this methodology, we identified several miRNAs and other sRNAs that predict transformation of MDS to AML. Importantly, this study was designed to identify these independent of increased blast percentage. Thus, these sRNAs are serving not as documentation of progression, but as true predictive markers of future transformation, providing the basis for the utilization of these sRNAs as biomarkers in MDS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Schematic of isomiRs using miR-181b-5p as an example. (B) Venn diagram showing the predicted mRNA targets for two isomiRs of miR-181b-5p and the parent miRNA.

Figure 2.

(A) Plot of the percentage blasts in the bone marrow (Y axis) in each of the study groups (X axis). (B-D) Scatter plots of expression of miR-181a-5p, miR-181b-5p, and miR-181d-5p across Groups A, B, and C.

Figure 3. Pathway schematic showing some of the key targets (in color) of the 5 miRNAs that differentiate Groups A and B. The AKT and MAPK pathways are redundantly regulated by these miRNAs.

Figure 4. tRNA alignment figure. This figure denotes the parent tRNA family type by color, and alignment position of tRNA (0-96) is presented as X-axis, and percent of reads aligned is presented as Y-axis.

Table 1**Patient Characteristics.**

	Group A	Group B	Group C
Number	19	11	10
Age (range)	64.2 (47-87)	64.1 (57-79)	60.3 (49-74)
Gender M:F	14M:5F	10M:1F	7M:3F
WBC (range)	4.93 (1.5-23.4)	4.25 (0.4-13.7)	4.79 (0.2-23.1)
HGB (range)	10.0 (8.7-12.2)	10.7 (8.9-14.3)	9.9 (8.5-12.8)
PLT (range)	147 (38-474)	104 (15-160)	106 (7-658)
% blasts BM (range)	3 (0-15.5)	8 (2-18)	36 (20-51)
Diagnosis (%)			
RCUD (MDS-SLD)	1 (5)	1 (11)	0
RARS (MDS-RS-SLD)	1 (5)	0	0
RCMD (MDS-MLD)	14 (74)	4 (44)	0
RAEB-1 (MDS-EB-1)	1 (5)	2 (22)	0
RAEB-2 (MDS-EB-2)	2 (11)	4 (44)	0
AML-MRC	0	0	10 (100)
Cytogenetics (%)			
normal	7 (34)	4 (44)	4 (40)
-5/del(5q)	0	0	0
-7/del(7q)	1 (5)	1 (11)	1 (10)
-Y only	1 (5)	0	0
-20,del(20q)	1 (5)	3 (33)	0
+8	1 (5)	0	1 (10)
complex (3)	6 (32)	3 (33)	2 (20)
other	2 (11)	0	2 (20)
Outcomes (%)			
Alive	5 (26)	0	2 ^{*#}
Deceased	13 (68)	11 (100)	7
Unknown	1 (5)	0	0

* two time points obtained from the same patient

on patient alive after stem cell transplantation, the second alive after going on clinical trial for an experimental agent

Abbreviations: WBC, white blood cell count; HGB, hemoglobin; PLT, platelets; BM, bone marrow; RCUD, refractory cytopenia with unilineage dysplasia (2008 World Health Organization,² WHO); MDS-SLD, myelodysplastic syndrome with single lineage dysplasia (2016 WHO³⁴); RARS, refractory anemia with ring sideroblasts (2008 WHO); MDS-RS-SLD, MDS with ring sideroblasts with single lineage dysplasia (2016 WHO); RCMD, refractory cytopenias with multilineage dysplasia (2008 WHO); MDS-MLD, myelodysplastic syndrome with multilineage dysplasia (2016 WHO); RAEB, refractory anemia with excess blasts (2008 WHO); MDS-EB, MDS with excess blasts (2016 WHO); F, female; M, male.

miRNA differential expression. Previous findings were predominantly from AML versus normal controls comparisons (see references in brackets).

Table 2

B vs A				C vs A				C vs B			
miRNA	Fold Change	Adjusted P	Previous Finding	miRNA	Fold Change	Adjusted P	Previous Finding	miRNA	Fold Change	Adjusted P	Previous Finding
miR-451a	-2.40	0.012	↓ ²²	miR-181a-5p	3.22	1.81E-07	↑ ^{14,49}	miR-29a-3p	-1.84	0.015	↓ ⁶⁸
miR-181a-5p	1.76	0.026	↑ ^{14,49}	miR-181b-5p	2.73	1.06E-05	↑ ^{14,49}				
miR-185-5p	-2.17	0.032	↑ ⁵⁰	miR-181a-3p	2.96	0.001	↑ ^{14,49}				
miR-181d-5p	2.37	0.032	↑ ^{14,49}	miR-181d-5p	2.56	0.004	↑ ^{14,49}				
miR-210-3p	-3.01	0.032	None	miR-215-5p	-5.33	0.004	None				
miR-486-5p	-2.60	0.037	↑ ⁶⁹	miR-222-3p	2.05	0.008	None				
miR-181b-5p	1.78	0.037	↑ ^{14,49}	miR-30a-5p	-2.17	0.008	None				
miR-93-5p	-1.90	0.037	↓ ²⁰	miR-10a-5p	3.43	0.010	↑ ⁷⁰				
miR-199b-5p	2.29	0.037	↓ ⁶¹	miR-196b-5p	3.42	0.010	↑ ⁷¹				
				miR-203a	-5.25	0.012	↓ ⁷²				
				miR-181c-5p	2.28	0.014	↑ ^{14,49}				
				miR-409-3p	-4.72	0.014	None				
				miR-340-5p	2.04	0.025	↑ ⁷³				

Table 3

IsomiR differential expression

B vs A			C vs A			C vs B		
IsomiR	Fold Change	Adjusted P	IsomiR	Fold Change	Adjusted P	IsomiR	Fold Change	Adjusted P
hsa-miR-10a-5p_+1	2.43	0.031	hsa-miR-181b-5p_+1	4.81	0.003	hsa-miR-7977_+1	-2.99	0.015
hsa-miR-92a-3p_+2	2.24	0.033	hsa-miR-92a-3p_+1	2.30	0.004	hsa-miR-144-5p_+1	3.98	0.015
			hsa-miR-378a-3p_+1	3.40	0.004	hsa-miR-7977_+2	-2.68	0.015
			hsa-miR-191-5p_+1	2.92	0.004	hsa-miR-30e-5p_+1	2.44	0.028
			hsa-miR-181c-3p_+1	4.20	0.004	hsa-miR-10a-5p_+1	2.54	0.039
			hsa-miR-126-3p_+1	4.04	0.004			
			hsa-miR-361-3p_+1	3.98	0.005			
			hsa-miR-223-3p_+1	3.65	0.005			
			hsa-miR-126-5p_+1	4.37	0.006			
			hsa-miR-10a-5p_+1	4.04	0.006			
			hsa-miR-140-3p_+1	2.33	0.014			
			hsa-miR-142-3p_+1	3.07	0.014			
			hsa-miR-30e-5p_+1	2.74	0.018			
			hsa-miR-28-3p_+1	2.68	0.018			
			hsa-miR-144-5p_+1	3.20	0.018			
			hsa-miR-320a_+2	3.20	0.018			
			hsa-miR-10a-5p_+2	3.38	0.018			
			hsa-miR-192-5p_+1	2.57	0.024			
			hsa-miR-142-5p_+1	2.81	0.024			
			hsa-miR-425-5p_+1	2.48	0.028			
			hsa-miR-133a-3p_+1	3.15	0.031			
			hsa-miR-1307-3p_+1	2.15	0.031			
			hsa-miR-142-3p_+2	3.70	0.031			
			hsa-miR-140-3p_+2	2.41	0.031			
			hsa-miR-146b-3p_+1	2.22	0.036			

Table 4

miRNAs that can distinguish between A and B independent of blast percentage.

miRNA	Coefficient	STDER	Adjusted p
hsa-miR-4865p	-0.0001	0.0000	0.04
hsa-miR-181a.5p	0.0010	0.0004	0.03
hsa.miR-181b.5p	0.0160	0.0058	0.03
hsa.miR-199b-5p	0.0636	0.0260	0.03
hsa-miR-181d-5p	0.2214	0.0851	0.03

Table 5

tRNA differential expression analyses by anticodon type.

B vs A			C vs A			C vs B		
Anticodon	Fold Change	Adjusted P	Anticodon	Fold Change	Adjusted P	Anticodon	Fold Change	Adjusted P
Asp	-2.46	0.001	Val	2.09	0.010	Phe	-3.76	9.30E-05
Leu	2.02	0.002	Gly	1.93	0.017	Ser	-1.91	0.001
Sup	2.69	0.011	His	1.83	0.022	Asp	1.99	0.006
Ser	1.53	0.031	Phe	-1.95	0.025	Leu	-2.18	0.006
Gln	2.11	0.045				Sup	-3.86	0.006
						Gly	1.77	0.012
						Cys	1.50	0.046