Expression Profiling and Functional Studies Reveal a Potential Role for microRNAs in Human Acute Myeloid Leukemia

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SUMMARY POINTS

- MicroRNA expression profiling can discriminate AML patients with distinct cytogenetic alterations (inv(16) or aberration of chromosome 7) and standard risk cytogenetics.
- Expression levels of specific miRNAs in standard risk patients correlate with various clinical markers and outcomes.
- miRNA expression alterations are linked to AML pathogenesis and may define a new class of biomarkers for diagnostic and/or prognostic applications.

INTRODUCTION

Acute myeloid lymphoma (AML) is a heterogenous disease of hematopoietic stem cells with substantially differential treatment outcome in individual patient groups. The differential course of the disease is associated with cytogenetic alterations that distinguish individual AML subpopulations. According to recently proposed model of leukemogenesis, the pathogenesis of AML is the consequence of at least two different molecular alterations targeting the physiological processes of differentiation and proliferation of hematopoietic precursor cells. Since there is now growing evidence that microRNA (miRNA) can affect various key cellular functions such as differentiation and proliferation control, it is reasonable to suspect a role for miRNAs in the pathogenesis of AML. The current study was designed to investigate 3 subsets of leukemia patients with precisely selected cytogenetic features to reduce the genetic complexity inherent to unselected AML patient cohorts.

MATERIALS AND METHODS

Blast and bone marrow samples were obtained from patients or healthy donors at the time point of diagnosis. Mononuclear cells were prepared by a standardized ficol procedure and cryopreserved in vials containing 5 to 20 x10^6 cells. miRNA isolation and profiling were performed as described in Shingara et al., RNA 2005. qRT-PCR were performed with the mirVana™ qRT-PCR Kit and Probe Sets (Ambion, Inc.) following the manufacturer instructions. In-vitro differentiation of hematopoietic stem cells was conducted essentially as described in Kiani et al., J. Leukoc. Biol. 2004. Pre-miR and Anti-miR molecules (Ambion, Inc.) were transfected in HeLa cells 48 hours prior the MTT or apoptosis assays were conducted as described in Illmer et al., Leukemia 2004.

RESULTS

Cytogenetic alteration	Age		Sex		FAB							Cytogenetics		Leukocytes		BM blasts	
	median	range	m	f	M0	M1	M2	M4	M5	M6	M7	sole	additional	median	range	median	range
Inv(16) n=12	37,5	(24-58)	10	2	0	0	0	12	0	0	0	7	5	68.75	(17.8-204)	64.50	(46-73)
SR n=21	41	(18-60)	15	6	0	12	1	2	6	0	0	14 (2)*	5	104.00	(3.9-380)	90.00	(80-96)
Abn. Chrom. 7 n=17	59	(18-77)	12	5	1	2	6	6	2	0	0	7	10	28.00	(2.4-120)	62.00	(35-94)

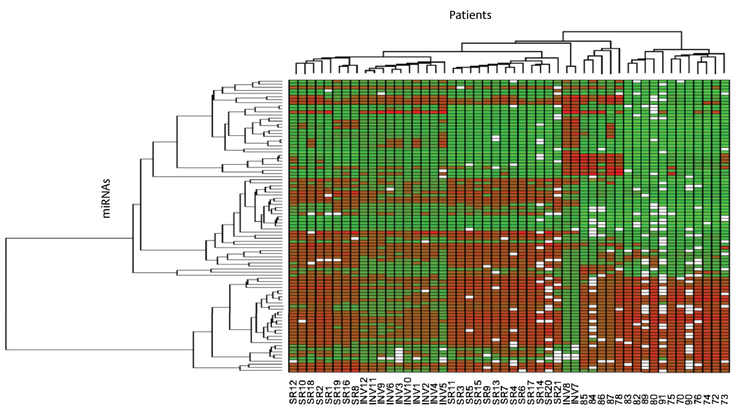


Figure 1: miRNA Expression Profiles in 50 AML Patient Samples
(Top) Clinical characteristics of the AML cohort studied. *Two patients were defined as SR AML by investigation with FISH analysis excluding high-risk cytogenetic features. (Bottom) Clustering analysis of 50 AML samples and 95 differentially expressed miRNAs. Hierarchical clustering using Euclidian distance and ANOVA test for the determination of differentially expressed miRNAs were done using the GEPAS v3.0 software. Arrays were median centered and normalized. Clusters were built with average linkage using Euclidian distance and correlation method.

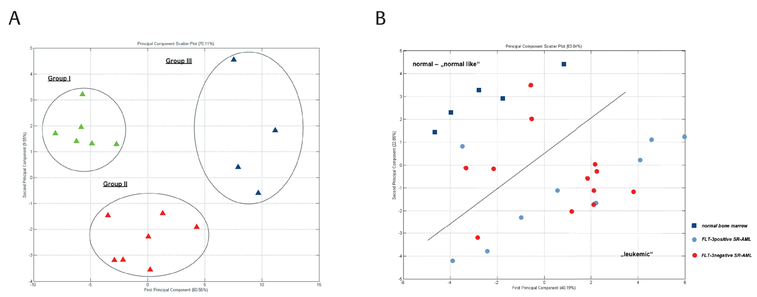


Figure 2: Principal Component Analysis of miRNA Expression in AML Samples
(A) Patients with aberrant chromosome 7 clustered in 3 distinct groups. (B) SR patients distribution as compared to normal bone marrow controls.

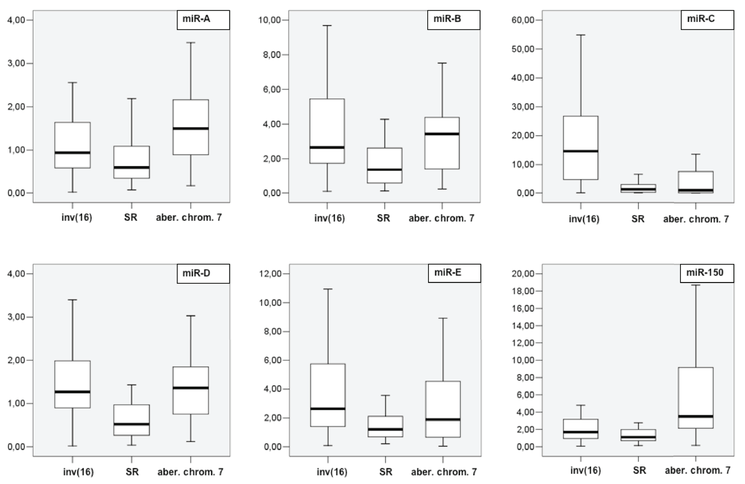


Figure 3: Quantitative Analysis of 108 AML Patient Samples by Real-Time qRT-PCR
qRT-PCR reactions were performed on 10-20 pg of RNA isolated from AML patients with inv(16) (n=33), SR cytogenetics (n=43) or aberrant chromosome 7 (n=32). Box plots were created using the SPSS system and compare fold expressions in patient classes relative to the median of 5 unselected normal bone marrow samples after normalization to 5S rRNA. miR-150 was previously shown to associate with immature hematopoietic cells (Monticelli et al., Genome Biol. 2005)

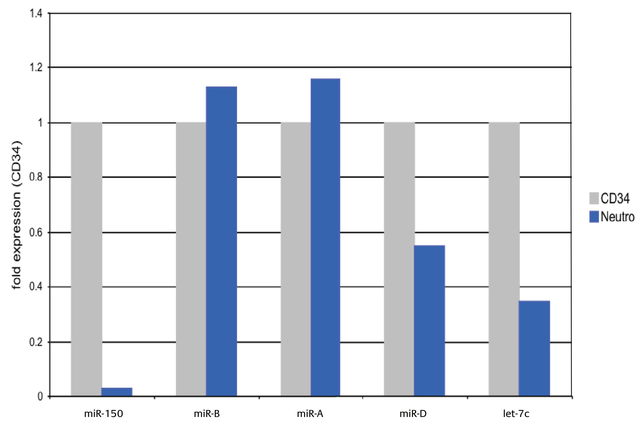


Figure 4: qRT-PCR Analysis of Selected miRNAs during Myeloid Differentiation
Freshly isolated CD34+ stem cells were differentiated in vitro towards the granulocyte lineage for 7 days. The graph compare relative qRT-PCR miRNA expression levels between days 0 (CD34) and 7 (Neutro).

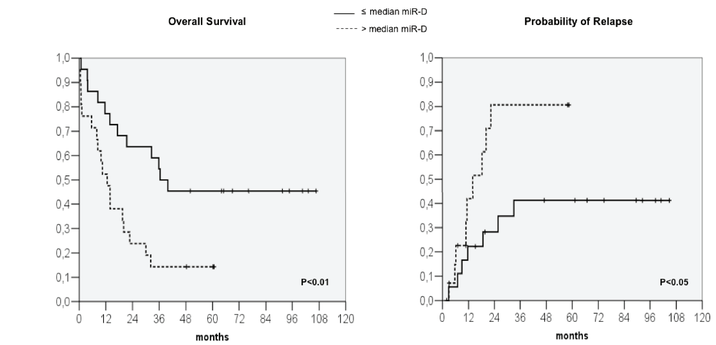
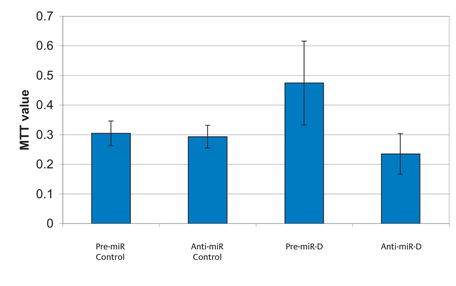


Figure 5: Prognostic Impact of miR-D Expression Level in SR AML Patients
Statistics on overall survival (left) and probability of relapse (right) in 43 AML patients with SR cytogenetics relative to the median expression level of miR-D were calculated by the method of Kaplan and Meyer. The p values are 2-sided and a significance level of 0.05 was used .



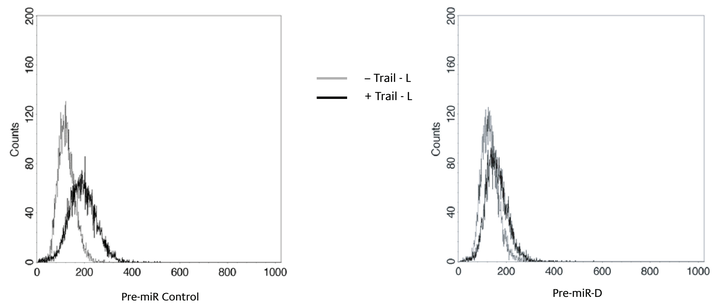


Figure 6: Functional Characterisation of miR-D in Cultured Cells
(Top) HeLa cells were transfected with the indicated synthetic RNA molecules. Twenty four hours post transfection, cells were treated with with 3ng/ml TRAIL-L and further incubated for 24 hours before performing the MTT proliferation assay. (Bottom) Same experiment as above with HeLa cells transfected with the indicated Pre-miR molecules and Tunel-FITC staining analyzed by FACS analysis (apoptosis assay).

CONCLUSION

miRNA expression profiling in blast samples from 50 AML patients with clearly defined molecular background revealed aberrant miRNA expression patterns in patients with loss of chromosome 7 material and standard risk cytogenetics. Subsequent qRT-PCR validation in 108 patient samples confirmed mis-expression of specific miRNAs.

Further statistical analyses on the SR patient cohort showed a positive or negative association of miRNA expression levels with various clinical markers or outcomes. For example, the expression level of miR-B correlated with the expression of the monocytic maturation marker CD14 and was negatively associated with the presence of high risk FLT-3 ITD (data not shown).

In addition, a multivariate model for likelihood of relapsing and overall survival showed that the expression level of miR-D had a highly significant negative prognostic value in SR AML patients. Since this miRNA could also be shown to modulate sensitivity to induced apoptosis in cultured cells, deregulated expression of miR-D may comprise a new mechanism for therapy resistance in SR AML patients.

In summary, these results suggest that miRNAs are involved in AML pathogenesis and may represent new targets for the development of therapeutic, diagnostic and/or prognostic strategies.