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ORIGINAL ARTICLE

The prognostic relevance of miR-212 expression with survival in cytogenetically and molecularly heterogeneous AML

SM Sun[1,4], V Rockova[1,2,4], L Bullinger[3], MK Dijkstra[1], H Döhner[3], B Löwenberg[1,5] and M Jongen-Lavrencic[1,5]

Acute myeloid leukemia (AML) is a highly heterogeneous disease, characterized by various cytogenetic and molecular abnormalities, many of which may express prognostic value. MicroRNAs (miRNAs) are a class of small regulatory RNAs. The prognostic value of miRNAs in AML is yet to be determined. Here, we set out to identify miRNAs that are consistent significant prognostic determinants, independent from other known prognostic factors. A discovery cohort ($n = 167$) and validation cohort ($n = 409$) of a heterogeneous AML population were used to reliably identify miRNAs with prognostic value. We report miR-212 as an independent prognostic factor, significantly associated with a prolonged overall survival (OS) and also event-free and relapse-free survival in a discovery cohort (hazard ratio (HR)s = 0.77, $P = 0.015$ for OS) that was subsequently confirmed in an independent validation cohort of 409 cases (HR = 0.83, $P = 0.016$). The prognostic significance and the prevalence of high miR-212 did not correlate with specific (cyto)genetic subtypes of AML. High miR-212 expression levels are associated with a gene expression profile that is significantly enriched for genes involved in the immune response. MiR-212 may improve the current prognostic risk stratification of mixed AML including normal karyotype AML and AML with cytogenetic and molecular abnormalities.

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INTRODUCTION

Acute myeloid leukemia (AML) is a complex heterogeneous disease, caused by a chain of events involving genetic and epigenetic changes. These alterations lead to a disease phenotype that is characterized by an accumulation of immature progenitors due to an increased proliferation and block in differentiation. Several of these molecular and genetic changes have been shown to have prognostic relevance and are useful for risk classification in treatment protocols.[1]

Currently, the panel of prognostic markers known in AML include clinical characteristics and cytogenetic abnormalities, such as t(8;21),[2,3] inv(16),[4] t(11q23)[5] as well as, molecular aberrations, including somatic gene mutations in nucleophosmin (*NPM1*),[6,7] CCAAT/enhancer-binding protein α (*CEBPA*),[8,9] internal tandem duplications in the FMS-like tyrosine kinase 3 gene (*FLT3*-ITD)[10] and DNA (cytosine-5)-methyltransferase 3 A (*DNMT3A*).[11–13] In addition, overexpression of particular genes, for example, high transcript levels of ecotropic virus integration site 1 (*EVI1*),[14] v-ets erythroblastosis virus E26 oncogene homolog (*ERG*), brain and acute leukemia, cytoplasmic *(BAALC*), meningioma (disrupted in balanced translocation) 1 (*MN1*) or cluster of designation 34 (*CD34)* have recently been proposed as prognostic biomarkers.[15–18]

MicroRNAs (miRNAs) are short noncoding RNAs (20–25 nt) capable of regulating protein levels by either cleavage of mRNA transcript or inhibition of translation.[19] There is an increasing body of evidence supporting the important role of miRNAs in hematopoiesis and cellular processes such as differentiation, proliferation and apoptosis.[20] Previously others and we have shown that different miRNA expression patterns reflect the cytogenetic and molecular heterogeneity of AML.[21–23] Recent findings have shown that several miRNAs have functional importance in the development of AML.[24–27] In a cohort of 122 AML patients, Garzon *et al.*[21] showed association of miR-199a, miR-199b, miR-191, miR-25 and miR-20 with adverse survival and were able to validate the association of miR-199 and miR-191 in an independent cohort of 60 AML patients. Marcucci *et al.*[23] reported the prognostic value of a miRNA signature in normal karyotype AML (NK-AML). Subsequently, in a cohort of 187 NK-AMLs, the expression of miR-181a, one of the miRNAs of the signature, predicted better survival.[28]

In the current study, we identified miR-212 in a discovery cohort ($n = 167$) as a prognostic relevant miRNA that is widely expressed in AML and that can be confirmed as predictor in an independent validation cohort of heterogeneous AML patients ($n = 409$). We report here that miR-212 expression positively correlates with survival, independently of known clinical, molecular and cytogenetic prognostic markers. Thus, the study reveals a profound independent prognostic impact of a single miRNA in a large heterogeneous cohort of AML including leukemia with normal karyotype as well as those with various cytogenetic and molecular abnormalities.

MATERIALS AND METHODS

Patients, treatment and mutational analysis

All patients included in this study had a newly diagnosed AML according the 2001 WHO classification. All patients provided written informed

[1]Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands; [2]Department of Biostatistics, Erasmus University Medical Center, Rotterdam, The Netherlands and [3]Department of Internal Medicine III, University of Ulm, Ulm, Germany. Correspondence: Dr M Jongen-Lavrencic, Department of Hematology, Erasmus University Medical Center, Dr Molewaterplein 50, 3015 GE, Rotterdam, The Netherlands.
E-mail: m.lavrencic@erasmusmc.nl
[4]These authors contributed equally to this paper.
[5]These authors contributed equally to this paper.
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consent in accordance with the declaration of helsinki and accord with assurance filled with and approved by the institutional review board of the Erasmus University Medical Center. The analysis was done in an initial discovery cohort consisting of 167 patients, treated according the protocols 04, 04A, 29 and 42 of the Dutch-Belgian-Hematology-Oncology-Cooperative group (HOVON; www.hovon.nl). The validation cohort originated from two different sources, that is, 183 patients of AML treated according to protocol HOVON-42 A and 226 AML patients treated according the AML German-Austrian Study Group (AMLSG) protocol 98 A.[29] The two cohorts showed similar distribution in miR-212 expression and some differences in patient characteristics. (Supplemental Figure 1, Supplemental Table 1). To increase power and to minimize differences, the latter two series were combined resulting in a validation cohort of 409 patients. Demographic, hematological and genetic features of the discovery cohort and validation cohort are given in Table 1. Both cohorts showed similar overall survival (OS; Supplemental Figure 2).

Samples, from either bone marrow aspirates or blood at the time of diagnosis, were purified with Ficoll-Hypaque (Nygaard, Oslo, Norway) centrifugation resulting in $>80\%$ of blast cells and subsequently cryopreserved.[9,30] Mutation analyses were performed as described previously.[7,9,31]

Table 1. Patients characteristics in the discovery and validation cohorts

	Discovery cohort		*Validation cohort*	
	(n = *167*)		(n = *409*)	
	No.	*(% Of cohort)*	*No.*	*(% Of cohort)*
Clinical parameters				
Age (mean)	43		44	
Range (min–max)	15	60	18	60
WBC, $\times 10^9$/l (mean)	54		45	
Range (min–max)	0.8	263.4	0.8	427
Sex				
Male	92	(55%)	205	(50%)
Female	75	(45%)	204	(50%)
ELN genetic risk				*(0%)*
Favorable	51	(31%)	143	(35%)
Intermediate-I	53	(32%)	128	(31%)
Intermediate-II	36	(22%)	69	(17%)
Adverse	27	(16%)	69	(17%)
Cytogenetics				
+8	12	(7%)	16	(4%)
−5 or −5q	0	(0%)	3	(1%)
−7 or −7q	7	(4%)	9	(2%)
−9q	4	(2%)	8	(2%)
11q23	5	(3%)	10	(2%)
t(6;9)	2	(1%)	2	(0%)
t(8;21)	9	(5%)	28	(7%)
t(9;11)	4	(2%)	9	(2%)
inv(3) or t(3;3)	0	(0%)	3	(1%)
inv(16)	9	(5%)	36	(9%)
Normal karyotype	84	(50%)	204	(50%)
Complex karyotype	14	(8%)	45	(11%)
Other	18	(11%)	37	(9%)
Molecular genetics[a]				
CEBPA single	4	(2%)	6	(1%)
CEBPA double	12	(7%)	20	(5%)
FLT3-ITD	39	(23%)	71	(17%)
FLT3-TKD	7	(4%)	28	(7%)
NPM1	49	(29%)	109	(27%)

Abbreviations: CEBPA, CCAAT/enhancer-binding protein α; ELN, European LeukemiaNet; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; NK-AML, normal karyotype acute myeloid leukemia; NPM1, nucleophosmin. [a]Molecular data determined in NK-AML.

RNA isolation, miRNA expression, data normalization and miRNA selection

After thawing total RNA isolation, quantitative RT-PCR and analysis were performed as described previously.[22,32]

Briefly, total RNA was extracted using Trizol according to manufacturer's protocol (Invitrogen, Breda, The Netherlands). The miR-212 expression in the discovery cohort was determined by real-time quantitative RT-PCR assays for miRNAs (Applied Biosystems, Nieuwekerk a/d IJssel, The Netherlands) in multiplex manner as described previously,[22] and for the validation cohort real-time quantitative RT-PCR was performed in a singleplex manner according to manufacturer's protocol. MiR-212 expression was determined centrally at one location and the validation cohorts from the two different trials showed similar distribution in expression. A shift in distribution was observed between the discovery and validation cohort, which correlates with the difference of single and multiplex RT-PCR reaction (Supplemental Figure 1).

Data was normalized using the endogenous control RNU48, which was found to be stably expressed in AML samples[22,32] and other cell types.[32–34] A minimal threshold was set for C_t values above 35 to a ΔC_t value of 16.5. The relative quantification method, $2^{-\Delta Ct\ 35}$ was used to calculate the expression relative to RNU48. Finally, the expression data was log transformed to obtain symmetrical distribution. The 167 cases of the discovery cohort were analyzed for gene-expression profiling using Affymetrix Human Genome U133 Plus 2.0 GeneChips (Affymetrix, Santa Clara, CA, USA) as described previously.[30] The gene expression data are available at http://www.ncbi.nlm.nih.gov/geo/ as accession GSE6891.

Definition of survival endpoints, molecular cytogenetic risk and statistical analysis

OS, event-free survival (EFS), relapse-free survival (RFS) and complete remission (CR) were defined according to recommendation of the European LeukemiaNet (ELN).[1] Genetic risk groups are reported according to the ELN criteria[1] with one minor adjustment, namely, instead of any mutation of *CEBPA* only double mutations in *CEBPA* were considered favorable. Favorable group includes inv(16)(p13.1q22), t(16;16)(p13.1;q22), t(8;21)(q22;q22), NK-AML with mutation in *NPM1* without *FLT3*-ITD[6,7] and NK-AML with *CEBPA* double mutation.[8,9] Intermediate-I refers to remainder of the NK-AML. Intermediate-II involves t(9;11)(p22;q23) and various other cytogenetic abnormalities not classified as favorable or adverse. Adverse risk designates inv(3)(q21q26.2) or t(3;3)(q21;q26.2), t(v;11q23), t(6;9)(p23;q34), −5 or 5q−, −7, abnl(17p) and complex karyotype.[1]

To test for the association to the various survival outcomes, that is, OS, EFS and RFS, the miRNA expression was used as a continuous variable in both univariable and multivariable analyses using Cox proportional hazards model (reported *P*-values correspond to the Wald test). The proportional hazards assumption was tested using scaled Schoenfeld residuals. To represent graphically the prediction capabilities of single miRNAs, we used Kaplan–Meier curves dichotomizing the expression based on the median expression value. Cox proportional hazards model with a penalized spline for miR-212 (df = 4) was fitted to assess, whether the miR-212 expression has a linear effect on the hazard function. The test for the nonlinearity (OS: $P = 0.28/0.6$ (univariable analysis, discovery/validation cohort) and $P = 0.18/0.69$ (multivariable analysis, discovery/validation cohort)) suggests that it is appropriate to treat the miR-212 expression as a continuous linear variable. Similar conclusion was obtained from an analogous analysis with EFS and RFS and a logistic regression model for CR. The shapes of the fitted spline curves did not indicate a better referral cutoff value than the median value. Log-rank test was then used to test for differences in survival distributions. Logistic regression was used to assess the association between the miRNA expression and CR, univariably and in a multivariable manner correcting for known prognostic markers (age, log(WBC) and ELN genetic risk). Treatment was added to the multivariable analysis to adjust for the differences in treatment protocols. Andersen–Gill model was used to model the association of survival in relation to a time-dependent covariate of transplantation. Spearman correlation analysis was performed to determine the association between two continuous variables, Wilcoxon rank sum test between a continuous and a categorical variable (two categories) and Fisher's exact test between two categorical variables.

To identify genes differentially expressed in miR-212 high/low groups (as determined by the median value), Wilcoxon rank sum analysis was performed, controlling for the false discovery rate (FDR) by the Benjamini–Hochberg procedure (FDR < 0.05). The expression values for every gene

Table 2. Association of miR-212 expression levels with achievements of complete response and survival in the discovery and validation cohorts

	Discovery cohort					*Validation cohort*				
	HR/OR	*s.e.*	z	P-*value*	*95% Confidence interval*	*HR/OR*	*s.e.*	z	P-*value*	*95% Confidence interval*
Univariable analysis										
CR	1.74	0.46	2.32	**0.020**	1.10–2.99	1.04	0.15	0.35	0.727	0.80–1.38
EFS	0.75	0.07	−3.14	**0.002**	0.59–0.89	0.86	0.06	−2.67	**0.008**	0.73–0.95
RFS	0.73	0.10	−2.55	**0.011**	0.52–0.92	0.82	0.07	−2.70	**0.007**	0.67–0.94
OS	0.78	0.08	−2.72	**0.007**	0.60–0.92	0.81	0.06	−3.36	**0.001**	0.69–0.91
Multivariable analysis[a]										
CR	1.78	0.48	2.03	<**0.001**	1.02–2.98	0.91	0.14	−0.67	0.501	0.67–1.21
EFS	0.78	0.08	−2.86	**0.004**	0.61–0.91	0.89	0.06	−1.87	*0.062*	0.76–1.01
RFS	0.77	0.10	−2.32	**0.020**	0.55–0.95	0.85	0.07	−2.07	**0.038**	0.70–0.99
OS	0.79	0.08	−2.44	**0.015**	0.62–0.95	0.85	0.06	−42	**0.016**	0.72–0.97

Abbreviations: CR, complete remission; EFS, event-free survival; HR, hazard ratio; OR, odds ratio; OS, overall survival; RFS, relapse-free survival. Univariable/multivariable logistic regression used for CR and Cox proportional hazards model for EFS, RFS and OS. HR >1 or <1 indicate an increased or decreased risk. OR >1 or <1 indicate increased or decreased odds for reaching CR. The sample sizes for OS and EFS of the discovery cohort and validation cohorts were $n=167$ and $n=409$, respectively. For RFS, the sample sizes were $n=141$ and $n=319$ for the discovery and validation cohorts, respectively. *P*-values <0.05 depicted in bold, *P* values <0.1 and >0.05 depicted in italic. [a]Multivariable Cox proportional hazard model adjusted for treatment, included known prognostic factors, that is, age, log(WBC) and molecular genetics (ELN genetic risk).

were determined from either a single probe or a combination of multiple probes. Only probes expressed above lower detection limit[30] in more than 20% of the cases were taken into account in the analysis. In case of multiple probes per gene, log-transformed MAS5 normalized expression intensities were combined by performing a principal component analysis using the first principal component. The expression values were then standardized to have zero mean and standard deviation one.

All performed statistical tests were two-sided. Survival and association analyses were done using Stata/Se v11.1 (College Station, TX, USA) and the gene expression data analysis was performed in R (version R-2.13.0). Ingenuity systems was used to determine overrepresented pathways in the selected genes.

RESULTS

High miR-212 expression is predictive for favorable survival

Using univariable analysis in the discovery cohort ($n=167$), we observed high miR-212 expression to be highly predictive for better OS (hazard ratio (HR) = 0.78, $P=0.007$). Higher miR-212 expression was also found to be strongly associated with higher CR rate, better EFS and RFS (Table 2). These findings were confirmed in the independent validation cohort ($n=409$), for OS (HR = 0.81, $P=0.001$), EFS and RFS. The relationship between high miR-212 and higher CR rate could not be reproduced in the confirmatory cohort (Table 2). Kaplan–Meier curves for OS (Figure 1), EFS and RFS (Supplemental Figure 3) depict the estimates of survival distribution in patients with high versus low miR-212 expression, as determined by a median expression value.

Independent prognostic value of miR-212

To correct for established prognostic factors, cytogenetic abnormalities and molecular mutations in the genes *NPM1*, *FLT3*-ITD according to ELN criteria and were included in the multivariable Cox proportional hazard model together with age and log(WBC). A minor modification was made with regards to the ELN criteria, normal karyotype with *CEBPA*, only double mutations were considered favorable instead of any mutated *CEBPA*. Difference in treatment protocols were taken along to adjust for treatment-related differences. The overall proportionality hazards assumption test was not significant (Supplemental Table 2). In the discovery cohort, high miR-212 remained highly significantly associated with better OS (HR = 0.79, $P=0.015$) as well as with the

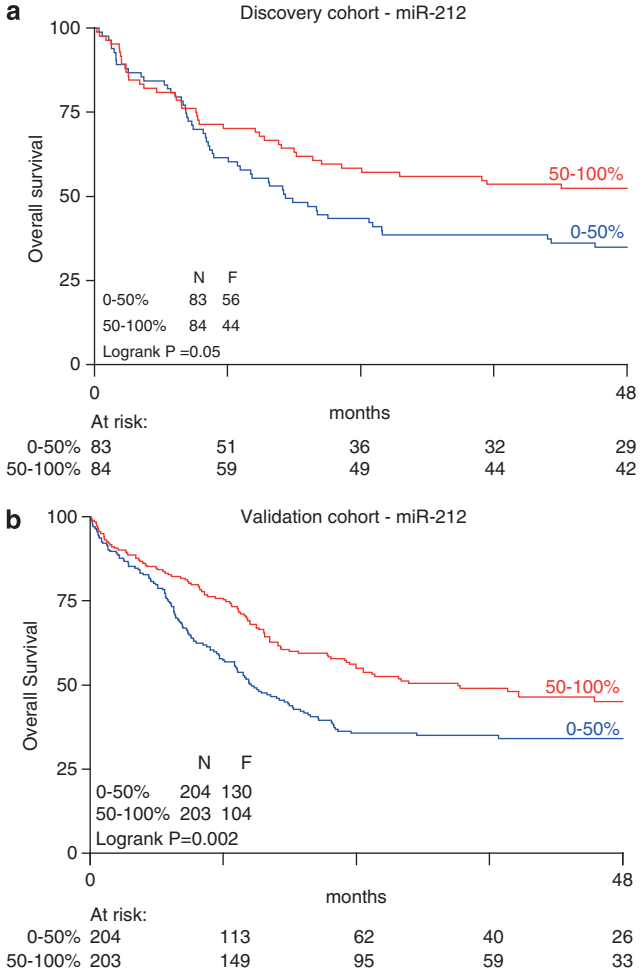

Figure 1. Kaplan–Meier survival curves of patients with high and low miR-212 expression. Patients were dichotomized into high miR-212 and low miR-212 groups based on median expression value of miR-212. Patients with high miR-212 expression had significantly better survival in both the discovery cohort (**a**) and validation cohort (**b**).

other survival endpoints, EFS and RFS. In the validation cohort, we were able to confirm the independent prognostic value of miR-212 for OS (HR = 0.85, $P = 0.016$; Table 2, Supplemental Table 2). Additionally, miR-212 was also significant as a favorable determinant of RFS in both the discovery (HR = 0.77, $P = 0.020$) and validation cohort (HR = 0.85, $P = 0.038$). It was borderline significant in the validation cohort for EFS (HR = 0.89, $P = 0.062$) (Table 2, Supplemental Table 2). The interaction term between miR-212 expression and the different genetic risk group was found insignificant for the discovery ($P = 0.733$) and validation cohort ($P = 0.320$; data not shown).

To determine whether the prognostic value of miR-212 is also independent of the allogeneic stem cell transplantation treatment that is known as a common factor to impact on relapse probability, we considered the Andersen–Gill model with transplantation as a time-dependent covariate for the discovery cohort. After addition of allogeneic transplantation in the model, miR-212 retained its significance (OS: HR = 0.77, $P = 0.014$; EFS: HR = 0.75, $P = 0.004$; RFS: HR = 0.70, $P = 0.009$).

Relationship of high miR-212 expression with other clinical and hematological features

The associations of the clinical and genetic characteristics with the expression levels of miR-212 in the discovery and validation cohorts are summarized in Table 3. Overall, there was no consistent relationship between the distribution of miR-212 expression levels and clinical parameters. Among the genetic subsets, only the presence of inv(16) was consistently associated with higher miR-212 in the discovery cohort and validation cohort.

Genes involved in immune response are enriched in patients with high miR-212 expression

To gain more biological insight in AML characterized by high miR-212 expression, we studied differential gene expression in the discovery cohort ($n = 167$). We found 867 genes/probes to be differentially expressed in between cases with high and low miR-212 expression (as determined by the median expression value; Supplemental Table 3). Analysis using ingenuity pathway analysis showed that most enriched pathways among the molecular and cellular functions belong to cell–cell interaction ($P = 5.44 \times 10^{-8}$), cell death ($P = 6.69 \times 10^{-8}$), antigen presentation ($P = 8.52 \times 10^{-8}$) and cellular movement ($P = 8.52 \times 10^{-8}$). At the level of physiological system development and function the most significantly enriched pathways were hematological system development and function ($P = 8.52 \times 10^{-8}$), hummoral immune response ($P = 8.52 \times 10^{-8}$), immune cell trafficking ($P = 8.52 \times 10^{-8}$) and hematopoiesis ($P = 3.64 \times 10^{-4}$) (Figure 2).

Strikingly, many of the genes in the differentially expressed genes between AML patients with high and low miR-212, in these enriched pathways, are involved in immune response, which largely was found upregulated in high miR-212 expression cases. Among genes involved in chemotaxis of immune cells are chemokine (C-C motif) ligand 3 (*CCL3*), chemokine (C-C motif) ligand 4 (*CCL4*) and chemokine (C-C motif) ligand 5 (*CCL5*). We identified other genes like *MN1*, *BAALC* and urokinase plasminogen activator receptor (*PLAUR*) being differentially expressed between AMLs with high and low miR-212 expression.

DISCUSSION

The relevance of miRNAs as prognostic markers in heterogeneous patient population of AML is largely unknown. In this study, we have investigated the role of miR-212 as prognostic factor in AML that are independent of established confounders and can be used as predictors for survival. In the present study, we were able to validate the strong prognostic value of miR-212 in an independent validation cohort.

Table 3. Relationship of miR-212 expression levels with clinical and genetic features of acute myeloid leukemia patients in the discovery and validation cohorts

	Discovery cohort (n = *167*)		*Validation cohort* (n = *409*)	
	P-*value*	*Median difference / Spearman correlation*	P-*value*	*Median difference / Spearman correlation*
Clinical parameters				
Age	0.345	$\rho = 0.073$	0.467	$\rho = -0.036$
WBC	0.695	$\rho = 0.030$	0.022	$\rho = -0.113$
Sex[a]	0.820	−0.043	0.179	−0.086
Cytogenetics				
+8	0.202	−0.093	0.250	0.192
t(8;21)	0.966	−0.053	0.642	−0.167
inv(16)	0.059	−0.501	0.004	−0.432
Normal karyotype	0.851	0.033	0.541	−0.060
Complex karyotype	0.961	0.103	0.009	0.220
Other	0.392	0.093	0.052	−0.279
Molecular genetics[b]				
CEBPA double	0.072	0.417	0.242	−0.280
FLT3-ITD	0.976	0.042	0.187	−0.433
NPM1	0.012	−0.060	0.684	0.212

Abbreviations: CEBPA, CCAAT/enhancer-binding protein α; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; NPM1, nucleophosmin. Table reports *P*-values of Spearman correlation test (together with Spearman correlation coefficients) or Wilcoxon rank sum test (together with median differences in positive vs negative groups) to assess the association between the miRNA expression and different clinical and molecular parameters for the subtypes consisting of more than 5% of the cohort. [a]Median difference between females vs males. [b]Molecular data only determined in patients with normal karyotype.

MiR-212 shows significant association with various survival endpoints using univariable and multivariable analysis including well-established prognostic confounders in two independent cohorts. Thus, miR-212 emerged from the analysis as a notably robust prognostic determinant of OS, EFS and RFS. MiR-212 expression level predicts better prognosis and adds to the prognostic effect of various previously established molecular and cytogenetic markers in a highly mixed population of AML. Previously, several other molecular prognostic markers have been identified in AML, such as mutations in the genes *NPM1*, *FLT3*-ITD, *CEBPA*, and high expression of the transcripts of *ERG*, *BAALC*, *CD34* and miR-181. However, these molecular biomarkers express prognostic impact that is mainly restricted to leukemia without cytogenetic abnormalities, so called NK-AML. Furthermore, a portion of these molecular markers like *ERG, BAALC, CD34* and *NPM1* show strong correlations with each other and or interact with other specific molecular subtypes.[15–18,28] The expression level of miR-212 does not associate with any particular known AML subtype. It presents an independent prognostic marker that may contribute to the current risk classification of AML patients. In addition, the present study involves patients of age 60 and younger; additional studies in independent cohorts with younger and elderly patients are warranted to determine the overall prognostic relevance of miR-212.

To derive some biological insights in AML characterized by high miR-212 expression, we identified genes differentially expressed

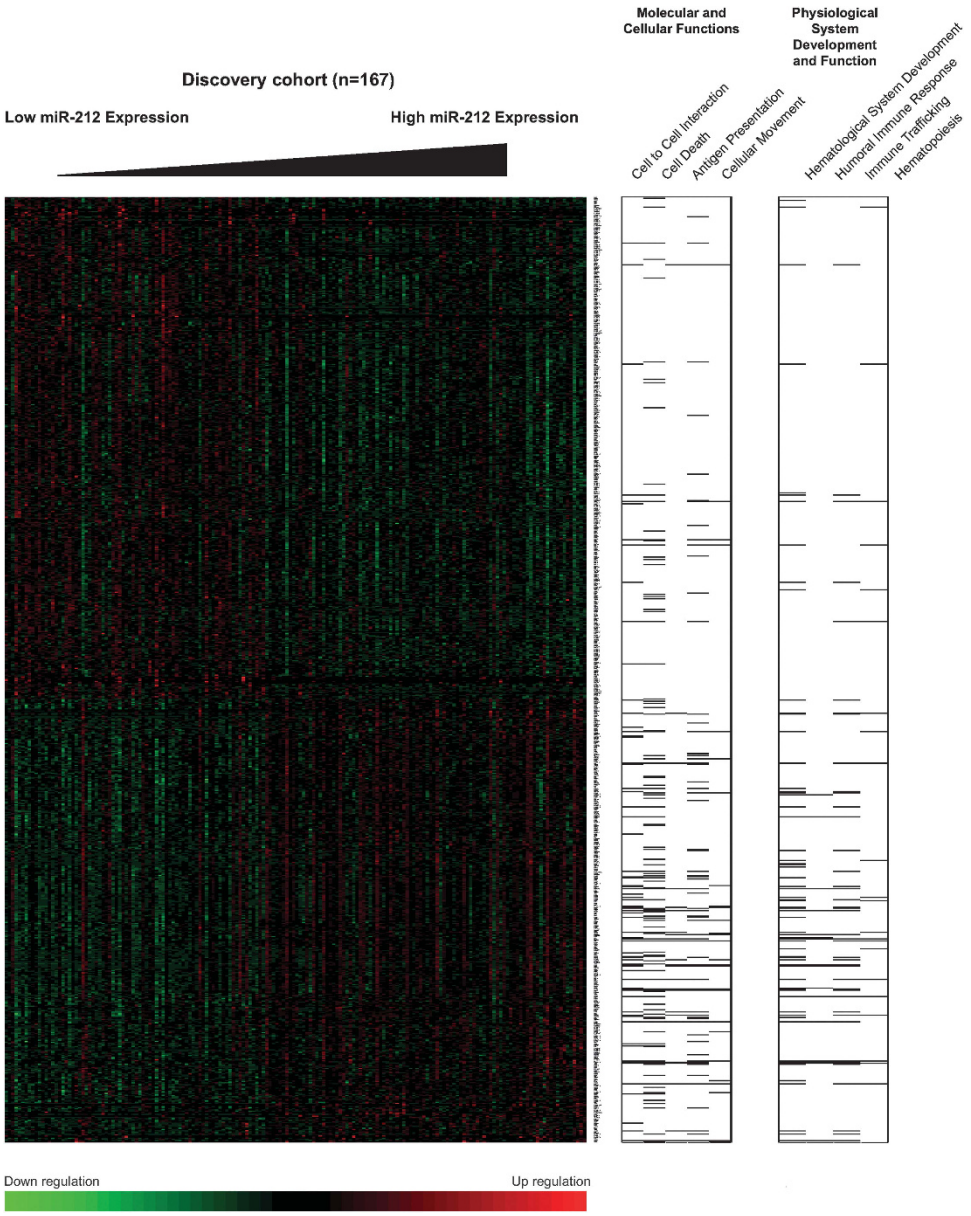

Figure 2. Differentially expressed probes between patients with high and low miR-212 expression in AML. The left panel shows a heatmap of 867 genes differentially expressed (FDR < 0.05) between high (above median) and low (below median) miR-212 expression groups (845 of these genes were also detected by Spearman correlation analysis (FDR < 0.05) using continuous miR-212 expression). Patients (columns) are ordered from the left to the right by increasing miR-212 values. Genes (rows) have been sorted according to their expression patterns by hierarchical clustering. Green color indicates expression values lower than the mean expression value (black) and red color indicates expression values higher than the mean expression value. The enriched pathways of the selected genes are depicted in the right panel. Columns represent top enriched pathways, where the black color indicates the involvement of the particular gene in the above mentioned category.

between patients with the highest and lowest miR-212 expression. Interestingly, we found a significant enrichment of genes involved in the immune response, in particular those involved in chemotaxis of immune cells. For example, *CCL3* and *CCL4*, upregulated in high miR-212 expression cases, belong to the CCL2-4/CXCL1/8 class of chemokines, and the release of these chemokines influence the T- and NK-cell chemotaxis.[36,37] Conceivably, stronger chemotaxis of immune cells by leukemic cells might contribute to their anti-leukemic effects as part of the immune response resulting in a better response to therapy in patients with high miR-212 expression.[38,39]

Among the differentially expressed genes, we also find genes with previously reported prognostic significance in AML. *BAALC*[15] and *MN1*[(ref. 16)] were downregulated and *PLAUR*[40] was upregulated in patients with high miR-212 expression. These markers were not included in the multivariable analysis. Following the addition of these factors to other previously indicated prognostic markers in the multivariable analysis, miR-212 remained significant indicating the prognostic ability of miR-212 independent of those molecular prognostic factors (Supplemental Table 4).

The function of miR-212 in AML and other hematopoietic malignancies is unknown. An increased expression of miR-212 was observed in pancreatic cancer where it has been suggested to act as an oncomiR by down modulation of retinoblastoma tumor suppressor.[41] In gastric cancer, miR-212 has been reported to be differentially expressed in certain subtypes with a possible

function in regulation of methylation via methyl-CpG-binding protein.[42–44] In head and squamous cell carcinoma and non-small cell lung cancer cell lines, miR-212 is shown to modulate the cell survival, either by downregulating *PED* and thereby causing an increase in tumor necrosis factor alpha-related apoptosis[45] or overcoming cetuximab resistance by targeting heparin-binding EGF-like growth factor.[46] This indicates the importance of miR-212 in regulating cell survival; the role of the aforementioned targets in AML is unclear as the targets are not among the genes found to be differentially expressed between patients with high and low miR-212 expression. Further research is required necessary to elucidate the biological role of miR-212 in AML.

In conclusion, we identified and validated that high expression of miR-212 predicts for better survival independently of other reported prognostic factors among molecularly and cytogenetically heterogeneous AML.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

SMS planned and carried out the experiments and analyzed the data. VR took part in the analysis. LB and HD provided the German data set and carried out experiments. MKD performed experiments. HD, BL and MJL designed the study and interpreted the results. SMS, VR, LB, HD, BL and MJL wrote or contributed to the manuscript.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)