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Expression and prognostic significance of hsa-miR-142-3p in acute leukemias

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Objectives: The microRNA 142 (miR-142) is expressed at high levels in mature hematopoietic cells and has a crucial role during T-lymphocyte development. Its role in leukemogenesis is unclear.

Patients and methods: Expression of miR-142 was analyzed in acute myeloid and lymphoblastic leukemia cells (*de novo*, cell lines). Data were compared to expression in CD34+ hematopoietic cells. Based on the miR-142 expression, clinical data such as overall survival was analyzed.

Results: MiR-142 expression in all leukemia cell lines and 86 % of the *de novo* samples was higher than in CD34+ cells. This difference could be detected in both, myeloid and lymphoid neoplastic cells. In AML patients with intermediate cytogenetic risk a high miR-142 expression was associated with a better overall survival.

Conclusions: MiR-142 expression in acute lymphoblastic as well as myeloid leukemia cells is higher than in CD34+ cells. Additionally, miR-142 expression might have prognostic relevance in AML-patients with otherwise an intermediate cytogenetic risk.

Key words: leukemia, prognostic factor, microRNA

Hematopoiesis is a highly regulated process and changes in DNA sequence, transcription or translation may lead to disorders such as leukemias. MicroRNAs (miRNAs, miR) are a class of small, non-coding RNA-molecules which influence the translation of mRNAs via the RNA interference pathway. The role of miRNAs in hematopoiesis and leukemia was reviewed by Yendamuri [1]. Some miRNAs are associated with the development of leukemias, such as the miR-15a and miR-16a which are involved in chronic lymphocytic leukemia (CLL) development [2]. More recently data on acute leukemias suggested a role of miRNAs in their leukemogenesis as well. Thus, it was possible to discriminate acute leukemias into myeloid (AML) or lymphoblastic (ALL) entities on the basis of miRNA expression profiles [3]. Additionally, AML-subtypes could be distinguished by means of their miRNA expression patterns and could be associated with different cytogenetic subgroups [4-5].

The *hsa-miR-142-3p* (miR-142) gene is located at chromosome 17q22. MiR-142 was first discovered to be involved in an aggressive B-cell leukemia with a t(8;17) translocation [6]. Landgraf *et al.* defined it as one of the five microRNAs that are highly specific for hematopoietic cells [7], and its expression is crucial for hematopoiesis in zebrafish [8]. The promoter of the miR-142 is negatively regulated by the transcription factor LIM domain only protein 2 (LMO2), which has crucial functions in hematopoiesis [9]. Apart of that, the mixed-lineage leukemia (MLL) methyltransferase binds to the promoter of the miR-142 [10]. Translocations involving the MLL-gene on chromosome 11 appear in about 5-6 % of AML and 7-10 % of ALL patients.

In the present study, we investigated the expression of miR-142 in leukemic cell lines, AML and ALL *de novo* samples and several mature hematopoietic cell populations of healthy donors by quantitative real-time PCR in relation to the expression in hematopoietic stem cells (HSC). Additionally, we analyzed survival of AML-patients in regard to their miR-142 expression.

Material and methods

De novo cells and cell lines. Hematopoietic stem cells (defined as CD34+/CD45+) were isolated from bone marrow of healthy volunteers. After ficoll density gradient centrifugation, cells were enriched for CD34+ cells using autoMACS™ separator (Miltenyi Biotec, Bergisch Gladbach, Germany). B-cells, monocytes and granulocytes were isolated from pooled blood of healthy volunteers. B-cells (CD19+/CD45+) and monocytes (CD14+/CD45+) were enriched using autoMACS™ separator.

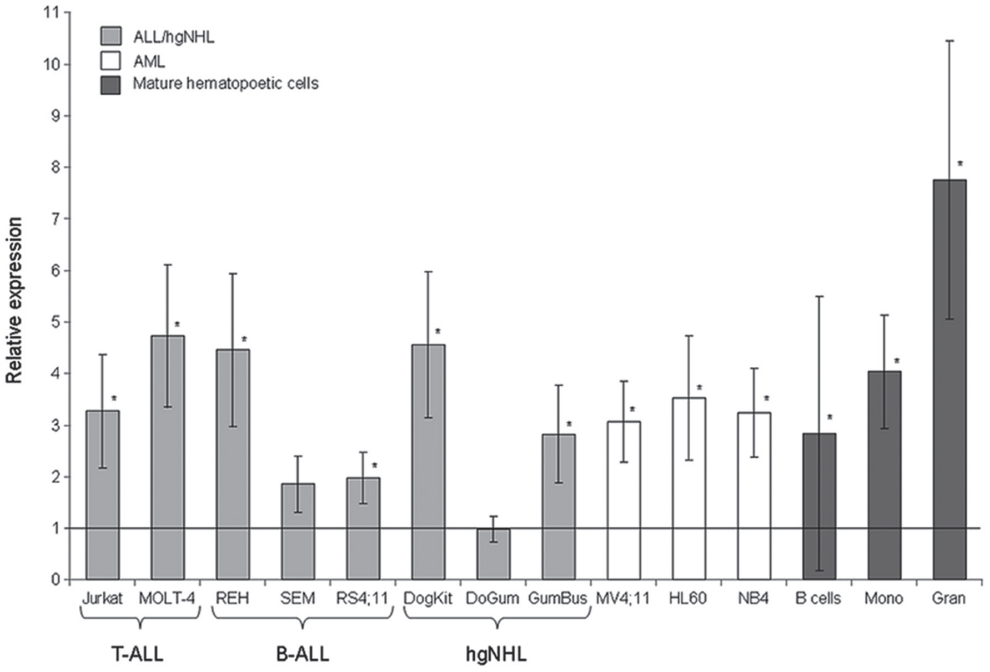

Figure 1. Expression of miR-142 in ALL/hgNHL cell lines (grey boxes), AML cell lines (blank boxes) and cells from healthy controls (black boxes, Mono = monocytes, Gran = granulocytes). Displayed is the relative expression compared to miR-142 expression in hematopoietic stem cells (CD34+) of healthy donors (set as 1, black line). * $p<0.05$

Cell purity was determined using flow cytometry. Purities over 70 % were achieved. Cells were lysed in Qiazol (Qiagen, Hilden, Germany) and stored frozen at – 80 °C for RNA isolation.

AML and ALL *de novo* samples were isolated from peripheral blood or bone marrow using density gradient centrifugation. Blast purity before PCR was ≥ 60 % (median 78.7 %, range: 60.0 – 99.6 %). Detailed information about patient characteristics including diagnosis and cytogenetic aberrations are listed in Table 1. No cytogenetic information was available for patients with Sézary Syndrome (No. 36 and 37).

Cell lines with different cytogenetic aberrations and phenotypes were used. The human cell lines SEM, REH, RS4;11 (all B cell precursor leukemias), Jurkat, MOLT-4 (both T-ALL), NB-4, MV4;11 and HL-60 (all AML) were cultured according to manufacturer's protocol. In addition, three high grade B-cell Non-Hodgkin's Lymphoma (hgNHL) cell lines were inquired. DogKit and GumBus were derived from patients with Burkitt Lymphomas, DoGum from a patient with a centroblastic NHL [11]. The media were supplemented with 10 % heat-inactivated fetal bovine serum (PAA, Pasching, Austria) and 1 % penicillin and streptomycin (Biochrom AG, Berlin, Germany). All cells were grown in a 37 °C and 5 % CO_2 humidified atmosphere incubator. The ALL and AML cell lines were purchased from DSMZ (Braunschweig, Germany). The hgNHL cell lines were kindly provided by G. Dölken, Department of Hematology and Oncology, University of Greifswald, Germany.

RNA isolation and quantitative real-time PCR. RNA was isolated from all samples using the miRNeasy Kit from Qiagen (Hilden, Germany) according to the manufacturer's protocol. Synthesis of cDNA and real-time PCR were preformed using TaqMan MicroRNA Assays from Applied Biosystems (Darmstadt, Germany). Total RNA was extracted from $2x10^5$ to $1x10^7$ cells. For first strand cDNA synthesis, 10 ng total RNA were used with 50 nm stem loop primer, 1x RT buffer, 50 U Multiscribe RT, 38 U RNase inhibitor in a final volume of 15 µl. The mix was incubated for 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C. Real-time PCR was performed using a standard TaqMan® protocol. The 20 µl PCR reaction contained 1.33 µl RT product, 1 x PCR MasterMix (Eurogentec, Cologne, Germany), 1 x TaqMan® probe/primer Mix (Applied Biosystems, Darmstadt, Germany). The reactions were incubated in a 96-well plate at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All PCR reactions were run in triplicate and included no template controls. RNA amount was normalized to the small nucleolar RNA c/d Box 48 (RNU48).

Flow cytometry. Expression of surface markers was analyzed by staining with fluorescence antibodies. Cells were washed twice (180 g, 10 min, 4 °C), resuspended in 100 µl PBS and 10 µl antibody was added. Cells were incubated on ice for 10 min and washed with the Lyse-and-wash-assistant (BD Biosciences, Heidelberg, Germany). The following antibodies were used: IgG1 FITC, CD2 FITC, CD45 FITC, IgG1 PE, CD3 PE, CD7 PE, CD19 PE, CD33 PE, CD34 PE (all from BD Bioscience, Germany). Flow cytometry analyses were performed using FACSCalibur (BD Bioscience, Germany) and data thus obtained were analyzed with CellQuest software (BD Bioscience, Germany).

Table 1. Patient characteristics

ID	Age	Sex	Diagnosis	Sample	Cytogenetic	Cytogenetic risk	Therapy	FLT3/ NPM1	Survivaltime [days]
01	53	m	AML M2	initial	47,XY,+8	intermediate	curative *	mut/n.d	249 †
02	56	f	AML M7	relapse	complex	high	curative *	wt/n.d.	800 †
03	72	m	AML M1	initial	46, XY	intermediate	curative	mut/mut	272 †
04	66	f	AML M1	initial	47,XX,+21	intermediate	curative	wt/mut	196
05	71	f	AML	initial	46,XX	intermediate	curative *	wt/wt	161 †
06	41	f	AML M2	initial	46,XX	intermediate	curative *	n.d./n.d.	210 †
07	68	m	AML M1	initial	46,XY	intermediate	curative	mut/n.d.	6 †
08	84	f	AML M5	initial	46,XX	intermediate	palliative	n.d./n.d.	2 †
09	79	f	AML	initial	46,XX	intermediate	palliative	wt/n.d.	76 †
10	70	m	AML M2	initial	47,XY,+8	intermediate	curative *	mut/wt	181 †
11	72	m	AML M4	initial	46,XY	intermediate	palliative	mut/wt	76 †
12	68	m	AML M2	initial	46,XY	intermediate	curative	wt/mut	464
13	75	f	AML M1	initial	complex	high	palliative	wt/wt	150 †
14	73	f	AML	initial	46,XX	intermediate	palliative	wt/mut	7 †
15	27	f	AML M4	initial	46,XX	intermediate	curative *	mut/wt	676
16	51	f	AML M3	initial	46, XX, t(15;17)(q22;q21)	low	curative	n.d./n.d.	33 †
17	64	m	AML M0	initial	46,XY	intermediate	curative *	wt/wt	268
18	45	m	AML	initial	46,XY	intermediate	curative	wt/mut	635
19	67	m	AML M5	initial	46,XX,t(4;11)(q21;q23)	intermediate	palliative	wt/wt	2 †
20	70	m	AML M1	initial	47,XY,+13	intermediate	palliative	n.d./n.d.	79 †
21	60	m	AML M0	initial	46,XY,del(12)(p11)	intermediate	curative *	mut/mut	182 †
22	70	f	AML M2	relapse	46, XX, del (5)(q13q33)	high	curative	n.d./n.d.	444
23	53	m	AML M4	initial	46,XY	intermediate	curative	wt/mut	81
24	74	f	AML M4	initial	46,XX	intermediate	palliative	n.d./n.d.	6 †
25	54	m	AML	relapse	46,XY,dic(1;22)(q10;q10)	intermediate	palliative	n.d./n.d.	112 †
26	86	m	AML M1	initial	45,XY,-7	high	palliative	n.d./n.d.	154 †
27	65	m	AML M2	initial	45,XY,-7	high	palliative	n.d./n.d.	44 †
28	69	m	AML M1	initial	46,XY	intermediate	palliative	n.d./n.d.	19 †
29	55	f	AML M2	initial	46,XX	intermediate	curative *	wt/wt	248 †
30	21	m	c-ALL	initial	47,XY,del(6)(q?16q23),+19,add(20)(p)	n.a.	curative	n.d./n.d.	50
31	76	f	c-ALL	relapse	46,XX,t(9;22)(q34;q11)	n.a.	curative	n.d./n.d.	287 †
32	41	f	c-ALL	initial	complex	n.a.	curative	n.d./n.d.	58
33	71	f	pre-B-ALL	initial	46,XX,t(4;11)(q21;q23)	n.a.	curative	n.d./n.d.	148
34	38	f	c-ALL	initial	46,XX,t(?;14)(?;32)	n.a.	curative	n.d./n.d.	348
35	26	m	T-ALL	initial	46,XY	n.a.	curative	n.d./n.d.	517

*m: male; f: female; complex: complex aberrant caryotype with 3 or more aberrations; curative: cytarabine and other anthracycline (doxorubicine, daunorubicine, idarubicine, mitoxantron); * allogeneic hematopoietic stem cell transplantation at some point; palliative= low dose chemotherapy (cytarabine or mitoxantrane) or supportive care only; wt: wildtype, mut: mutated, n.d.: not done; † dead; n.a.: not applicable*

Statistical analysis. Relative quantification of miRNA expression was calculated with the Relative Expression Software Tool© [12, 13]. MiR-142 Expression of hematopoietic stem cells was used as calibrator. A p-value less than 0.05 was considered to be significant. Survival analysis and Kaplan-Meier plots were generated using SPSS 15.0.

Results

MiR-142 expression in leukemic cell lines and mature hematopoietic cells. Expression of miR-142 was detected in all investigated cell lines and mature hematopoietic cells (Figure 1). Granulocytes, monocytes and B cells from healthy donors displayed a significant higher expression of miR-142 than HSC. Granulocytes showed a 7.8 fold higher, monocytes a 4.0 fold higher and B cells a 2.8 fold higher expression than HSC, respectively.

The T-ALL cell lines Jurkat and MOLT-4 showed a significant higher miR-142 expression than HSC (3.3 fold higher in Jurkat, 4.7 fold higher in MOLT-4, respectively). The pre-B-ALL cell line REH showed a 4.5 higher expression, the cell lines RS4;11 and SEM a 2.0 and 1.9 higher expression than HSC. The miR-142 expression in REH and RS4;11 was significantly different from miR-142 expression in HSC. All three AML cell

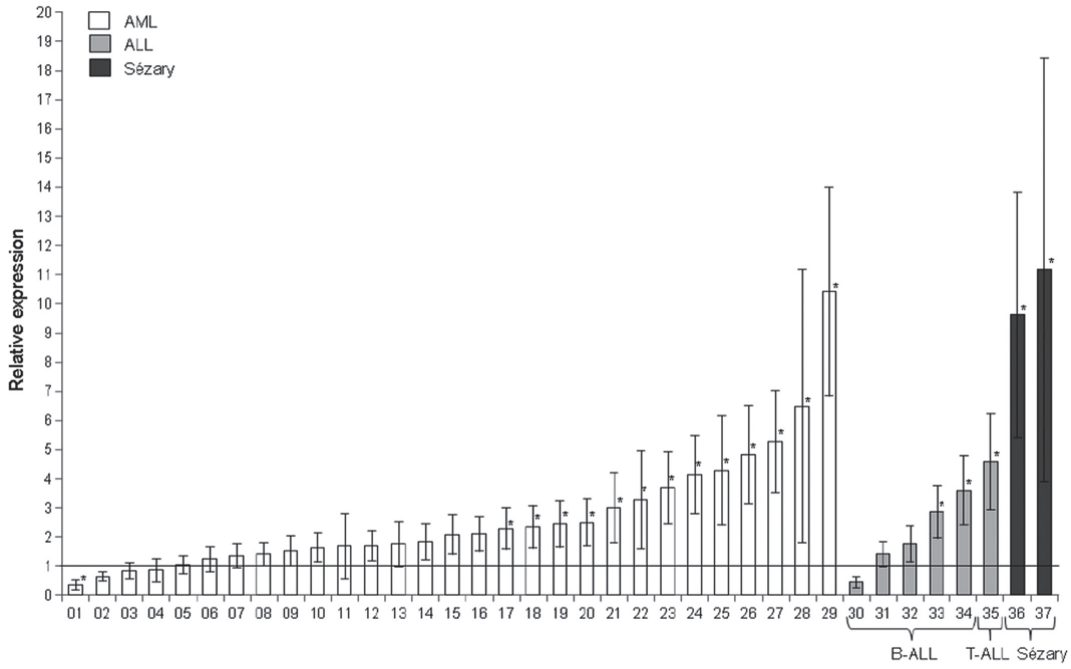

Figure 2. Expression of miR-142 in AML (blank boxes, n=29), ALL (grey boxes, n=6) and Sézary (black boxes, n=2) *de novo* cells. Displayed is the relative expression compared to miR-142 expression in hematopoietic stem cells (CD34+) of healthy donors (set as 1, black line). * p<0.05

lines showed a higher miR-142 expression compared to HSC, but the differences between the cell lines were only small. The highest miR-142 expression was detected in the HL-60 cell line (3.5 fold), followed by NB4 (3.2 fold) and MV4;11 (3.0 fold). No clear association of miR-142 expression and immature vs. mature leukemia subtypes could be identified.

MiR-142 expression in AML and ALL de novo samples. A total of 29 AML samples were analyzed (Table 1). 26 samples were taken at initial diagnosis, three were collected at relapse. Seventeen patients were treated with a curative intention, twelve patients received a palliative therapy. Classification of the cytogenetic risk in AML patients was performed according to the MRC-AML-10 study [14] and resulted in the following groups: low risk (n=1), intermediate risk (n=23) and high risk (n=5). Additionally, six ALL samples, including four common ALL, one pro-B-ALL, one T-ALL, and two samples with Sézary syndrome were analyzed.

In 86 % of the AML patient samples (25/29) the expression was higher than in HSC. In thirteen cases this difference was significant (Figure 2). Four samples had a lower miR-142 expression compared to HSC, one value being significantly lower. We could not identify a correlation between miR-142 expression and age at diagnosis or morphologic subtype (data not shown).

All three T-cell samples (T-ALL and Sézary) showed a very high miR-142 expression compared to HSC (4.6 fold to 11.2 fold higher). One B-ALL sample showed a lower miR-142 expression, four showed a higher miR-142 expression than HSC.

The cell lines MV4;11, RS4;11 and SEM and the patient samples 19 and 33 had a translocation involving chromosome 4 and the MLL-methyltransferase gene on chromosome 11. Compared to the other samples without this translocation, we saw no obvious differences in the miRNA-142 expression.

Prognostic significance of miR-142 expression in AML cells. We than investigated whether the miR-142 expression correlated with the clinical course of the patients. The samples taken at initial diagnosis from patients receiving a potential curative therapy were grouped according to the miRNA-expression, forming two groups with either a high or a low expression using the median expression as cut-off. A Kaplan–Meier survival analysis showed no differences between the groups (p=0.346) (Figure 3A). Next, we analyzed the survival in a more homogenous subgroup, i.e. only the patients with an intermediate cytogenetic risk and a curative therapy. Again, the median expression of this cohort was used as cut-off (Table 2). In this analysis, the patients with high miR-142 expression had a significant better prognosis compared to the patients with low miR-142 expression (p=0.032) (Figure 3B), indicating that in this subgroup miR-142 expression might serve as a prognostic marker.

Discussion

During the past years, intensive studies have linked the expression of miRNAs to the development of different diseases including cancer [7]. Apart of regulation mechanisms of

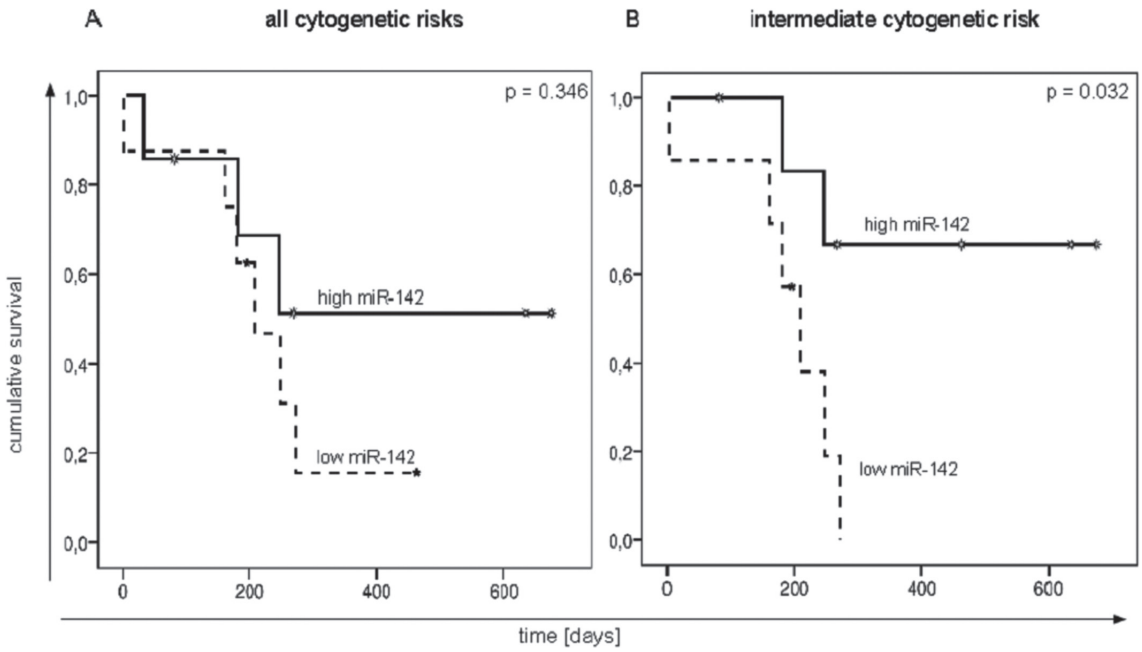

Figure 3. Overall survival of AML patients based on their miR-142 expression in AML blasts at initial diagnosis is displayed. All patients received a potentially curative therapy. In figure 3A all AML patients irrespective of their cytogenetic risk were analyzed (n=15, high: n= 7, low: n=8). In figure 3B only AML patients with an intermediate cytogenetic risk were analyzed (n=14, high: n= 7, low: n=7).

miRNA expression, cytogenetic aberrations might play a role in miRNA deregulations.

Here, we studied the expression of miR-142 in leukemic cell lines, AML and ALL *de novo* samples and several mature hematopoietic cells in relation to the expression in HSC. Subsequently, we analyzed survival of AML-patients in regard to their miRNA-expression.

Mature hematopoietic cells showed a higher miR-142 expression than immature hematopoietic cells. This is in line with published expression data showing a higher expression in mature cells compared to immature cells [7]. A current model for miRNA-dependent regulation of hematopoiesis [1] suppose a crucial role for the miR-142 in T-cell development, but the role of miR-142 in myeloid development is currently less clear.

Our results showed the highest miR-142 expression in mature granulocytes. Interestingly, the transcription factor LMO2, which plays a crucial role in miR-142 transcription by inhibiting miR-142 expression [8], is regulated itself negatively by the miR-223 [15]. This miRNA is known to be involved in granulopoiesis and is expressed at high levels in mature granulocytes. As LMO2 negatively regulates miR-142 expression, but is itself inhibited by miR-223, it is consequent to find a high miR-142 expression in cells expressing miR-233 at high levels like granulocytes.

Neilson *et al.* investigated the miRNA expression in different stages of T cell development [16]. They found a variation of miR-142 expression between different maturation states with the highest expression in mature CD4 positive cells. The

Table 2. Expression of miR-142 and survival time of patients with initial diagnosis, potential curative therapy and intermediate cytogenetic risk

Expression lower than Median			Expression higher than Median		
ID	***MiR-142 expression***	***Survival time [d]***	***ID***	***MiR-142 expression***	***Survival time [d]***
01	0.36	249 †	12	1.71	464
03	0.85	272 †	15	2.09	676
04	0.86	196	17	2.30	268
05	1.06	161 †	18	2.35	635
06	1.24	210 †	21	3.01	182 †
07	1.37	6 †	23	3.69	81
10	1.65	181 †	29	10.42	248 †

†: *dead*

investigated T-ALL cell lines Jurkat and MOLT-4 showed both a higher miR-142 than HSC. The expression in Jurkat (3.3 fold) is a little lower than in MOLT-4 (4.7 fold). This might be due to a deletion of chromosome 17 in Jurkat cells, the *miR-142* gen locus. However, miR-142 expression in Jurkat is more than half of the expression in MOLT-4, indicating a possible over expression of the remaining *miR-142* gene to compensate the deletion. The three T-cell samples (ALL and Sézary) showed the highest miR-142 expression of all ALL samples. This might be due to a potential deregulation of the transcription factor LMO2, which is found in about 60 % of T-ALL samples [17]. Further investigation with a coinstantaneous analysis of miR-142 and LMO2 expression in a larger cohort are required to prove this link.

The two B-ALL cell lines RS4;11 and SEM harbor a t(4;11)(q21;q23) translocation involving the *MLL* gene. The cell line REH harbors a translocation t(12;21). To date only one study investigated the miRNA expression in ALL samples in regards to MLL mutational status [18]. They showed a lower miR-142 expression in MLL mutated samples, however these differences were not significant. This is in line with our results. An interpretation of miR-142 expression in B-ALL *de novo* samples is so far difficult to make due to the small, heterogeneous group.

All three AML cell lines showed a higher miR-142 expression compared to HSC, but the differences between the cell lines were only small (Figure 1). To our knowledge, this is the first investigation that analyses the miR-142 expression in these cell lines in relation to HSC. Previous publications using Northern Blot, microarrays or qPCR with absolute quantification showed a different order of expression [19, 20], presumably due to the different sensitivities of the used methods. Our finding is in some regards unexpected, as HL60 has a hypotretraploid karyotype (4n; 82-88) with loss of two chromosomes 17. Nevertheless HL60 has a higher miR-142 expression as NB4 with a third chromosome 17 and MV4;11 which has no translocation involving this chromosome. Accordingly, there seem to be a different regulation of miR-142 expression in these cell lines.

In 86 % of the AML patient samples the expression was higher than in HSC. Only four samples had a lower miR-142 expression compared to HSC. We could not identify a correlation between miR-142 expression and morphologic subtype (data not shown). Garzon *et al.* showed a higher expression of miR-142 in AML patients with mutations in the NPM1 gene [21]. This could not be confirmed in our study. Nine of 19 patients with NPM1 analyses had NPM1 mutations but did not differ in regards to their miR-142 expression (data not shown).

Several studies have linked the expression of certain miRNAs to the clinical outcome. For example, the expression of the miRs 191 and 199a correlated with event free and overall survival in AML patients [5]. Here, we could demonstrate an influence of miR-142 expression as prognostic marker for a small but homogenous group of AML patients (initial diagnosis, potential curative therapy and intermediate cytogenetic risk). A high miR-142 expression correlated with a better overall survival in this subgroup. This is the first investigation showing a potential role of miR-142 expression as prognostic marker. Interestingly, so far most prognostic implications for microRNAs are associated with a better survival for low miRNA-expression in contrast to the above shown results that indicate that a high miR-142 expression is associated with a better survival.

In conclusion, our analysis showed a variable expression of miR-142 in leukemic cells and patient samples. In most cases expression was higher (25/29 AML patients, 7/8 ALL patients, all cell lines, granulocytes, monocytes and B cells) compared to HSC, which might reflect the higher maturation status of these cells. Moreover, in our cohort miR-142 expression was a prognostic marker within the AML intermediate cytogenetic risk group as AML patients with a high miR-142 expression in their blasts showed a survival benefit compared to patients with low miR-142 expression. We therefore conclude that miR-142 expression might have prognostic relevance in AML.

References

[1] YENDAMURI S, CALIN GA. The role of microRNA in human leukemia: a review. Leukemia. 2009; 23: 1257–63. http://dx.doi.org/10.1038/leu.2008.382

[2] CALIN GA, CIMMINO A, FABBRI M, FERRACIN M, WOJCIK SE et al. MiR-15a and miR-16-1 cluster functions in human leukemia. Proc Natl Acad Sci U S A. 2008; 105: 5166–71. http://dx.doi.org/10.1073/pnas.0800121105

[3] MI S, LU J, SUN M, LI Z, ZHANG H et al. MicroRNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Proc Natl Acad Sci U S A. 2007; 104: 19971–6. http://dx.doi.org/10.1073/pnas.0709313104

[4] DEBERNARDI S, SKOULAKIS S, MOLLOY G, CHAPLIN T, DIXON-MCIVER A, et al. MicroRNA miR-181a correlates with morphological sub-class of acute myeloid leukaemia and the expression of its target genes in global genome-wide analysis. Leukemia. 2007; 21: 912–6.

[5] GARZON R, VOLINIA S, LIU CG, FERNANDEZ-CYMERING C, PALUMBO T et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood. 2008; 111: 3183–9. http://dx.doi.org/10.1182/blood-2007-07-098749

[6] GAUWERKY CE, HUEBNER K, ISOBE M, NOWELL PC, CROCE CM. Activation of MYC in a masked t(8;17) translocation results in an aggressive B-cell leukemia. Proc Natl Acad Sci U S A. 1989; 86: 8867–71. http://dx.doi.org/10.1073/pnas.86.22.8867

[7] LANDGRAF P, RUSU M, SHERIDAN R, SEWER A, IOVINO N et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell. 2007; 129: 1401–14. http://dx.doi.org/10.1016/j.cell.2007.04.040

[8] NISHIYAMA T, KANEDA R, ONO T, TOHYAMA S, HASHIMOTO H et al. miR-142-3p is essential for hematopoiesis and affects cardiac cell fate in zebrafish. Biochem Biophys

Res Commun. 2012 Sep 7;425(4):755-61. http://dx.doi.org/10.1016/j.bbrc.2012.07.148

[9] YUAN W, SUN W, YANG S, DU J, ZHAI CL et al. Down-regulation of microRNA-142 by proto-oncogene LMO2 and its co-factors. Leukemia. 2008; 22: 1067–71. http://dx.doi.org/10.1038/sj.leu.2405001

[10] GUENTHER MG, JENNER RG, CHEVALIER B, NAKAMURA T, CROCE CM et al. Global and Hox-specific roles for the MLL1 methyltransferase. Proc Natl Acad Sci U S A. 2005; 102: 8603–8. http://dx.doi.org/10.1073/pnas.0503072102

[11] BRACHT K, KIEFER T, DOLKEN G, BEDNARSKI PJ. Characterization of three B-cell lymphoma cell lines from chemotherapy resistant patients with respect to in vitro sensitivity to 21 antitumor agents, ABC-transporter expression and cellular redox status. J Cancer Res Clin Oncol. 2007; 133: 957–67. http://dx.doi.org/10.1007/s00432-007-0241-x

[12] PFAFFL MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001; 29: e45. http://dx.doi.org/10.1093/nar/29.9.e45

[13] PFAFFL MW, HORGAN GW, DEMPFLE L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002; 30:e36. http://dx.doi.org/10.1093/nar/30.9.e36

[14] GRIMWADE D, WALKER H, OLIVER F, WHEATLEY K, HARRISON C et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood. 1998; 92: 2322–33.

[15] FELLI N, PEDINI F, ROMANIA P, BIFFONI M, MORSILLI O et al. MicroRNA 223-dependent expression of LMO2 regulates normal erythropoiesis. Haematologica. 2009; 94: 479–86. http://dx.doi.org/10.3324/haematol.2008.002345

[16] NEILSON JR, ZHENG GXY, BURGE CB, SHARP PA. Dynamic regulation of miRNA expression in ordered stages of cellular development. Genes Dev. 2007; 21: 578–89. http://dx.doi.org/10.1101/gad.1522907

[17] SHARMA VM, DRAHEIM KM, KELLIHER MA. The Notch1/c-Myc pathway in T cell leukemia. Cell Cycle. 2007; 6: 927–30. http://dx.doi.org/10.4161/cc.6.8.4134

[18] SCHOTTE D, CHAU JC, SYLVESTER G, LIU G, CHEN C et al. Identification of new microRNA genes and aberrant microRNA profiles in childhood acute lymphoblastic leukemia. Leukemia. 2008; 23: 313–22. http://dx.doi.org/10.1038/leu.2008.286

[19] KASASHIMA K, NAKAMURA Y, KOZU T. Altered expression profiles of microRNAs during TPA-induced differentiation of HL-60 cells. Biochem Biophys Res Commun. 2004; 322:403–10. http://dx.doi.org/10.1016/j.bbrc.2004.07.130

[20] MERKEROVA M, BELICKOVA M, BRUCHOVA H. Differential expression of microRNAs in hematopoietic cell lineages. Eur J Haematol. 2008; 81: 304–10. http://dx.doi.org/10.1111/j.1600-0609.2008.01111.x

[21] GARZON R, GAROFALO M, MARTELLI MP, BRIESEWITZ R, WANG L et al. Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proc Natl Acad Sci U S A. 2008; 105: 3945-50. http://dx.doi.org/10.1073/pnas.0800135105