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1 **Distinctive microRNA expression profiles in CD34+ bone marrow cells from patients**  
2 **with myelodysplastic syndrome**

3

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10 **Running title:** MiRNA expression profiles in MDS

11

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23 **KEYWORDS**

24 myelodysplastic syndrome, miRNA expression, CD34+ cells, microarrays, 14q32 miRNA

25 cluster

1 **ABSTRACT**

2 MicroRNAs (miRNAs) are small non-coding RNAs functioning as regulators of  
3 hematopoiesis. Their differential expression patterns have been linked with various  
4 pathological processes originating from hematopoietic stem cells (HSCs). However, limited  
5 information is available regarding the role of miRNAs in myelodysplastic syndrome (MDS).  
6 Using miRNA arrays, we measured expression of 1,145 miRNAs in CD34+ bone marrow  
7 cells obtained from 39 MDS and acute myeloid leukemia (AML) evolved from MDS patients,  
8 and compared them to those of 6 healthy donors. Differential miRNA expression was  
9 analyzed and a panel of up-regulated (n=13) and down-regulated (n=9) miRNAs was found  
10 ( $p < 0.001$ ) in MDS/AML patients. An increased expression of a large miRNA cluster mapped  
11 within 14q32 locus was detected. Differences in miRNA expression of MDS subtypes showed  
12 distinction between early and advanced MDS; an apparent dissimilarity was observed  
13 between RAEB-1 and RAEB-2 subtypes. In early MDS, we monitored up-regulation of pro-  
14 apoptotic miR-34a which may contribute to the increased apoptosis of HSCs. Patients with  
15 5q- deletion were characterized by decreased levels of miR-143\* and miR-378 mapped within  
16 the commonly deleted region at 5q32. This is an early report describing differential  
17 expression in MDS CD34+ cells, likely reflecting their disease-specific regulation.

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## 1 INTRODUCTION

2 Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal preleukemic conditions  
3 with high risk of transformation into secondary acute myeloid leukemia with multilineage  
4 dysplasia (AML with MLD) occurring in approximately 30-40% of MDS cases. Individuals  
5 with MDS exhibit blood cytopenias, ineffective hematopoiesis and usually hypercellular or  
6 normocellular bone marrow (BM)<sup>1</sup>. Progression of the disease is assumed to be a multi-step  
7 process of acquisition of several successive genetic abnormalities in a normal hematopoietic  
8 stem cell (HSC) which ultimately leads to malignant transformation and expansion of the  
9 MDS clone<sup>2</sup>. According to the World Health Organization (WHO) classification system,  
10 MDS is divided into several subtypes with different characteristics and worsen prognoses: 5q-  
11 syndrome, refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS),  
12 refractory cytopenia with multilineage dysplasia (RCMD) and refractory anemia with excess  
13 blasts (RAEB-1 and RAEB-2)<sup>3</sup>.

14 MicroRNAs (miRNAs) are small (~21nt) endogenous non-coding RNAs that negatively  
15 regulate gene expression of complementary mRNAs. Many crucial cellular processes are  
16 under control of miRNAs whose deregulation is implicated in the disease development and/or  
17 progression<sup>4</sup>. Only a limited number of reports investigating miRNA deregulation in MDS  
18 has been published to date<sup>5-9</sup>. Pons *et al.*<sup>5</sup> measured gene expression of 25 mature miRNAs in  
19 mononuclear cells (MNCs) isolated from bone marrow (BM) and peripheral blood of MDS  
20 patients. The authors reported an over-expression of miRNA cluster miR-17-92 in MDS and  
21 differential expression of miR-15a and miR-16 between low-risk and high-risk subgroups of  
22 patients. Hussein *et al.*<sup>9</sup> measured expression profiles of 365 miRNAs in total BM cells and  
23 demonstrated up-regulation of miR-1/miR-133a cluster. These studies examined miRNA  
24 expression in unsorted blood cells; however, gene expression profiles remarkably differ  
25 between various cell types<sup>10</sup>. Thus, increase of the blast count in high-risk MDS influences

1 miRNA levels when measured in whole blood samples. Therefore, separation of one cell type  
2 is essential for accurate gene expression profiling. Since MDS originates from HSC, we  
3 believe the testing of CD34+ fraction is of primary interest.

4 To understand impact of miRNA deregulation on MDS development and progression, we  
5 performed miRNA microarray analysis on purified CD34+ BM cells obtained from patients  
6 with MDS and AML evolved from MDS. Additionally, we profited from our previous gene  
7 expression profiling study<sup>11</sup> and associated miRNA levels with the appropriate gene  
8 expression data.

9

## 10 **DESIGN AND METHODS**

### 11 **Patients**

12 This study comprised 43 BM samples from Czech patients with primary MDS and AML  
13 evolved from MDS. According to the WHO 2001 classification criteria, patients' diagnoses  
14 were as follows: 5q- syndrome (n=6), RA (n=4), RARS (n=1), RCMD (n=6), RAEB-1 (n=7),  
15 RAEB-2 (n=10), and AML with MLD (n=9). Patients' age ranged between 31-79 years  
16 (average 63) and the female/male distribution was 16/27. The clinical characteristics of all  
17 patients are summarized in Table 1 and their karyotypes in supplementary Table 1. BM  
18 samples obtained from 9 healthy donors were used as controls. Their age ranged between 20-  
19 40 years (average 30) and the female/male distribution was 3/6. All tested subjects signed the  
20 informed consent and Institutional Review Board approved the study.

21

### 22 **CD34+ cell separation**

23 MNCs were separated from BM samples by Ficoll-Hipaque density gradient centrifugation  
24 (GE Health Care, Little Chalfont, United Kingdom). CD34+ cells were isolated from MNCs  
25 using magnetic cell separation according to the manufacturer's instructions (Direct CD34

1 Progenitor Cell Isolation MACS Kit, Miltenyi Biotec, Bergisch Gladbach, Germany). The  
2 purity of isolated cells (>95%) was tested using FACS Aria (Becton Dickinson, San Jose, CA,  
3 USA).

4

#### 5 **RNA extraction**

6 Total RNA was isolated by the method of Chomczynsky and Sacchi<sup>12</sup> and subsequently  
7 treated with DNase I (Qiagen, Hamburg, Germany). Final RNA integrity was evaluated using  
8 Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The RNA integrity number  
9 (RIN) was found to be 9.1 on average and RNA quality was comparable within the different  
10 samples.

11

#### 12 **MiRNA microarray profiling**

13 Human v2 MicroRNA Expression Profiling Kit (Illumina, San Diego, CA, USA) was used for  
14 measurement of gene expression of 1,145 human miRNAs (including 246 novel miRNA  
15 candidates denoted by HS codes; annotation file available at  
16 <http://www.switchtoi.com/annotationfiles.ilmn>). Only samples with sufficient RNA yield (39  
17 samples of MDS/AML with MLD and 6 control samples, input 200ng of total RNA) were  
18 hybridized on Illumina 12-sample BeadArrays. Scanning was performed on the BeadStation  
19 500 instrument (Illumina).

20

#### 21 **Microarray data analysis**

22 Quality control, background subtraction and quantile normalization of the raw data were done  
23 by GenomeStudio software (Illumina). Only probes which reached detection P-value < 0.01  
24 were included for further analyses. Logarithmic transformation of the data was done to obtain  
25 normal linear distribution. Subsequent analyses were performed using MeV v4.3.2 software<sup>13</sup>.

1 Welch approximate t-test was applied to find out differentially expressed miRNAs between  
2 two groups of samples: (i) patients and controls, and (ii) patients with and without del(5q).  
3 Analysis of variance (ANOVA) was performed using a 1% false discovery rate threshold to  
4 identify differentially expressed miRNAs between different MDS subtypes. Hierarchical  
5 clustering of miRNA expression data was done using average linkage and Euclidean distance.  
6

### 7 **Quantitative real-time polymerase chain reaction (qRT-PCR)**

8 The microarray results were confirmed in the complete set of samples (43 samples of  
9 MDS/AML with MLD and 9 controls) by qRT-PCR using RotorGene 3000 apparatus  
10 (Corbett Research, Sydney, Australia). qRT-PCR was performed with following validated  
11 TaqMan MicroRNA Expression Assays (Applied Biosystems, Foster City, CA, USA): miR-  
12 10a (assay ID 000387), miR-34a (000426), miR-323-3p (002227) and miR-378 (000567).  
13 Resulting data were normalized to the endogenous control RNU48 and relative miRNA  
14 expression levels were calculated by the  $\Delta\Delta C_T$  method. Statistical analyses were performed  
15 using GraphPad Prism 4 software (GraphPad Software, La Jolla, CA, USA).

16

### 17 **MiRNA target prediction**

18 Putative miRNA targets were predicted by MicroCosm Targets v.5  
19 (<http://www.ebi.ac.uk/enright-srv/microcosm/>), TargetScan 5.1 (<http://www.targetscan.org/>)  
20 and PicTar (<http://pictar.mdc-berlin.de/>) software.

21

## 22 **RESULTS**

### 23 **MiRNAs differentially expressed between patients and healthy individuals**

24 MiRNA expression profiles were measured in CD34+ cells isolated from BM of patients with  
25 MDS, AML with MLD and healthy donors (for normalized microarray data see

1 supplementary Table 2). Out of 1,145 tested miRNAs, 371 miRNAs were not detected and  
2 were excluded from further analyses. Subsequently, several statistical tests were applied on  
3 the filtered data set to identify differentially expressed miRNAs.

4 We defined a set of 22 miRNAs differentially expressed between patients and controls  
5 ( $p < 0.001$ ): 13 miRNAs were up-regulated (miR-299-3p, miR-299-5p, miR-323-3p, miR-329,  
6 miR-370, miR-409-3p, miR-431, miR-432, miR-494, miR-654-5p, miR-665, HS\_40 and  
7 HS\_206) and 9 miRNAs were down-regulated (miR-196a\*, miR-423-5p, miR-525-5p, miR-  
8 507, miR-583, miR-940, miR-1284, miR-1305 and HS\_122.1) in patients (hierarchical cluster  
9 in Figure 1). 9 up-regulated miRNAs (miR-299-3p, miR-299-5p, miR-323-3p, miR-370, miR-  
10 409-3p, miR-431, miR-432, miR-494 and miR-654-5p) mapped within 14q32 miRNA cluster.  
11 Also other miRNAs from this cluster displayed trend of increased expression in approx. 80%  
12 of MDS/AML patients (data in suppl. Table 2).

13

#### 14 **MiRNA expression profiles in MDS subtypes**

15 To determine miRNA expression differences between MDS subtypes, we grouped the patients  
16 into five categories: 5q- syndrome, low risk MDS, RAEB-1, RAEB-2 and AML with MLD.  
17 Patients with RA (n=1), RARS (n=1) and RCMD (n=6) formed together the “low-risk MDS”  
18 group due to a small number of samples. Using one-way ANOVA test, we identified a set of  
19 45 differentially expressed miRNAs with respect to the disease subtypes. Hierarchical  
20 clustering performed for the deregulated miRNAs resulted in the determination of five distinct  
21 clusters (Figure 2). MiRNAs grouped in red cluster showed slightly higher expression in low-  
22 risk MDS and RAEB-1 than in 5q- syndrome and high-risk patients. On the contrary,  
23 miRNAs in green cluster were down-regulated in low risk MDS and REAB-1 and displayed  
24 increased or normal levels in the other samples. MiRNAs in blue and brown cluster were  
25 down-regulated only in low-risk MDS category. Further, we detected 4 transcripts (miR-422a,



1 miR-617, HS\_63 and HS\_305\_b; violet cluster) whose expression correlated with disease  
2 progression; their expression was low in normal samples and early MDS and high in  
3 advanced MDS and AML with MLD with elevation of their expression level between RAEB-  
4 1 and RAEB-2.

5

#### 6 **MiRNAs deregulated in patients with 5q- deletion**

7 Our MDS subject cohort included 6 patients with 5q- syndrome and 6 patients with advanced  
8 MDS bearing 5q- deletion (RAEB-1\_3, RAEB-2\_3, RAEB-2\_4, RAEB-2\_9, RAEB-2\_10,  
9 and AML with MLD\_6). A non-parametric t-test was applied to identify miRNAs  
10 differentially transcribed in patients with and without deletion in the 5q32 region. Expression  
11 levels of 13 miRNAs were elevated and 7 of them were reduced in the patients with the  
12 deletion ( $p < 0.01$ , Figure 3).

13 Figure 4 shows expression of miRNAs located in deleted region in 5q- syndrome (miR-143,  
14 miR-145, miR-146a and miR-378), demonstrating that only expression of miR-378 and miR-  
15 143\* was significantly reduced in the patients with 5q- deletion.

16

#### 17 **Validation of microarray data**

18 Using qRT-PCR, expression of 4 miRNAs was measured in the complete set of samples (43  
19 patients and 9 controls): miR-10a, miR-34a, miR-323-3p, and miR-378 (Figure 5). We  
20 observed differences between miRNA levels in particular disease subtypes comparable to  
21 those obtained by microarrays. Moreover, level of miR-10a inversely associated with  
22 progression of the disease (miR-10a level was increased in 5q- syndrome and RA/RARS  
23 samples and reduced in RCMD, advanced MDS and secondary AML compared to healthy  
24 donors).

25

## 1 **miRNA targets**

2 To identify target genes of miR-10a, miR-34a, miR-378 and miR-127, we searched within  
3 previously published studies and also predicted new putative miRNA targets with potential  
4 implication in MDS (Table 2). We attempted to correlate miRNA expression levels with those  
5 of their targets measured in our previous study<sup>11</sup>. However, we confirmed inverse association  
6 of only a small number of target genes: increase of miR-34a corresponded to 70% reduction  
7 of *SPII* transcript level in early MDS (p=0.04), reduction of miR-378 to 30% increase of  
8 *SUFU* level in 5q- samples (p=0.01) and miR-127 up-regulation to 100% reduction of *BCL6*  
9 level in all MDS (p=0.02).

10

## 11 **DISCUSSION**

12 Microarrays have been anticipated to bring new information to the current knowledge of  
13 molecular pathogenesis of MDS. Several studies focused on gene expression signatures of this  
14 disorder<sup>11,14,15</sup>; however, not many crucial questions have been answered. These studies  
15 identified many deregulated genes whose alteration may be critical to the MDS phenotype but  
16 no fundamental implications like new targets for therapeutic intervention or improvement of  
17 prognostic scoring system have been revealed. Recently, our laboratory performed another  
18 gene expression profiling of CD34+ progenitors in patients with MDS and secondary AML<sup>11</sup>,  
19 and the miRNA profiling study presented here continues with further characterization of the  
20 same cohort of patients. We expected that the integration of mRNA and miRNA expression  
21 data might provide a more complex insight into the disease pathogenesis.

22 MiRNA expression analysis was done on CD34+ cells obtained from 39 patients (MDS and  
23 AML with MLD) and 6 controls. We identified a set of 22 miRNAs differentially expressed  
24 between patients and controls. Interestingly, 9 of the up-regulated miRNAs were located at  
25 14q32. Up-regulation of several miRNAs from this region was observed also in AML bearing

1 t(15;17) translocation<sup>16</sup>. 14q32 comprises 40 miRNA genes with imprinted expression  
2 controlled by a distant differentially methylated region (DMR)<sup>17</sup>. In our study, we detected  
3 significant over-expression of the whole cluster in the majority of MDS/AML patients. We  
4 hypothesized that the cluster up-regulation might be caused by altered imprinting in DMR  
5 possibly due to uniparental disomy (UPD) and/or deregulated epigenetic mechanisms in  
6 14q32. We read through recent publications whether any defect was detected in this region;  
7 however, no copy number variations or UPD found in 14q32<sup>18,19</sup> suggests rather a change in  
8 the methylation or acetylation status or aberrant characteristics of some transcriptional  
9 regulators. Limited information is available about the function of 14q32 miRNAs; however,  
10 several publications suggested their implication in cell development and oncogenesis. For  
11 example, miR-127, a member of 14q32 miRNA cluster, is involved in B-cell differentiation  
12 process through post-transcriptional regulation of *BLIMP1* and *XBPI*<sup>20</sup>. In MDS, we detected  
13 negative association of expression of another miR-127 target, proto-oncogene *BCL6*<sup>21</sup>,  
14 functioning also as a transcriptional regulator required for B- and T-lymphocyte terminal  
15 differentiation<sup>22</sup>. Thus, over-expression of miR-127 may be linked with the down-regulation  
16 of genes involved in B-cell lineage differentiation previously seen in MDS<sup>11,23</sup>.

17 In CD34+ cells of all MDS subtypes, we confirmed up-regulation of miR-1/miR-133a cluster  
18 ( $p < 0.05$ , see supplementary Table 2), which was recently published by Hussein et al.<sup>9</sup> In  
19 contrast to increased expression in MDS/AML, this miRNA cluster was down-regulated in  
20 neutrophils from patients with polycythemia vera and essential thrombocytosis<sup>24</sup>, indicating its  
21 different roles in myeloid neoplasms.

22 5q- syndrome is a MDS subtype characterized by interstitial deletion of the long arm of  
23 chromosome 5. The commonly deleted region (CDR) at 5q32 contains 40 genes and several  
24 of them may be implicated in hematopoiesis but none of the genes seems to be responsible for  
25 all characteristics of 5q- syndrome<sup>25-27</sup>. Three miRNAs (miR-143, miR-145, and miR-378) are

1 mapped within the region and miR-146a is located in its proximity. Expression of these  
2 miRNAs was examined by Starczynowski *et al.*<sup>28</sup> who showed correlation between del(5q)  
3 and the loss of miR-145 and miR-146a in a murine model. However, in human CD34+ and  
4 unsorted BM cells any expression changes of miRNAs encoded in CDR were not detected<sup>7,9</sup>.  
5 Our data suggested down-regulation of miR-378 and miR-143\* (complementary to miR-143  
6 but less abundant) whereas the level of miR-145 and miR-146a remained at the control level.  
7 In addition, 50% decrease of miR-378 and miR-143\* expression corresponded with the  
8 haploinsufficiency status. The discrepancy in the results is probably caused by small numbers  
9 of samples in the studies and should be clarified in future.

10 We hypothesize that an altered epigenetic mechanism at the remaining chromosome, perhaps  
11 as a compensational event, may explain why deletion at 5q32 does not lead to reduced  
12 expression of the presented miRNAs. Interestingly, we found specific down-regulation of  
13 miR-143\* but not of miR-143 in 5q- syndrome. Expression of different miRNA variants is  
14 usually tissue-specific with lower expression of the complementary variants. It is generally  
15 thought that these minor variants are not so important for mRNA regulation. Therefore, 5q-  
16 deletion might be compensated only in the case of major variant of miR-143.

17 In 5q- patients, we observed significant reduction of miR-378 level. However, validation of  
18 the microarray data revealed rather its non-specific deregulation. miR-378 seems to be  
19 affected either directly (through chromosome 5 rearrangement as a part of complex  
20 karyotype) or indirectly (through deregulation of some upstream target) also in high-risk  
21 MDS. This miRNA promote cell survival, tumor growth and angiogenesis by targeting *SUFU*  
22 gene expression<sup>29</sup>. Our previously published gene expression data<sup>11</sup> showed an increase of  
23 *SUFU* transcript level in 5q- syndrome patients. Another putative target of miR-378 is  
24 transcription factor *ETV6*. This gene is involved in a large number of chromosomal  
25 rearrangements associated with leukemia, including MDS<sup>30,31</sup>. Since *ETV6* is required for

1 hematopoiesis, it may be candidate gene generating MDS phenotype. Thus, our findings  
2 suggest that the reduction of miR-378 expression may be an event contributing to the  
3 pathogenesis of MDS.

4 Our data provided evidence for deregulation of miRNAs in particular MDS subtypes.  
5 Interestingly, an apparent dissimilarity was observed between RAEB-1 and RAEB-2  
6 expression patterns. The miRNA profile of RAEB-1 patients was more similar to controls and  
7 early MDS subtypes whereas RAEB-2 profiles rather resembled those of secondary AML,  
8 suggesting transformation of the disease as early as between RAEB-1 and RAEB-2.

9 Expression of miR-10a seems to negatively associate with progression status of MDS  
10 patients. Deregulation of miR-10a in various myeloid disorders has been previously reported  
11 (down-regulation in AML unsorted BM cells<sup>16</sup>, down-regulation in chronic myeloid leukemia  
12 CD34+ cells<sup>32</sup> or up-regulation in BM MNCs of MDS patients<sup>5</sup>), indicating its different  
13 tissue-specific roles in myeloid neoplasms. MiR-10a regulates translation of *HOX* genes<sup>33</sup> that  
14 play important roles in regulation of early stages of hematopoiesis, including self-renewal of  
15 HSCs<sup>34</sup>. Thus, reduction of miR-10a level in advanced MDS may induce (through up-  
16 regulation of *HOX* genes) conversion of BM HSCs to leukemic cells, and its decrease might  
17 be a marker of MDS progression.

18 Further, we detected statistically significant over-expression of miR-34a in early MDS  
19 (approx. 10-fold,  $p < 0.001$ ) and its level was also non-significantly increased in AML with  
20 MLD. MiR-34a is a direct pro-apoptotic transcriptional target of p53<sup>35</sup> and may play, by  
21 targeting *BCL2*<sup>36</sup>, a role in the increased apoptosis of BM progenitors seen in low-risk MDS.

22 In addition, we observed an inverse association between expressions of miR-34a and its  
23 putative target *SPI1* in early MDS. *SPI1* encodes transcription factor PU.1, mediating myeloid  
24 cell development<sup>37</sup>, whose expression is down-regulated in particular subtypes of AML and  
25 MDS<sup>38,39</sup>. Taken together, our results suggest that the up-regulation of miR-34a in early MDS

1 possibly affects not only apoptosis of HSCs through *BCL2* but could also decrease PU.1  
2 expression, which in consequence may impede myeloid signaling and contribute to ineffective  
3 hematopoiesis of early MDS.

4 In this study, we attempted to integrate miRNA expression data with the data from gene  
5 expression profiling previously performed in our laboratory<sup>11</sup>. However, we confirmed only a  
6 limited number of miRNA/mRNA interactions. It is known that miRNAs rather suppress  
7 target gene translation, not reflecting its effect on mRNA level. This regulatory impact of  
8 miRNAs at the post-transcriptional level may be reason why mRNA microarrays did not fully  
9 meet the expectations of finding new genes eligible for targeted therapy of MDS. We believe  
10 the mRNA expression profiling represents preliminary data for future proteomic approaches  
11 that will monitor effector molecules, and thus study the real situation in the cell.

12 This study identified miRNAs whose expression is deregulated in all MDS/AML with MLD  
13 patients and several miRNAs specific for particular disease subtypes. This is an early report  
14 describing complete miRNA expression profiles of MDS CD34+ cells measured by miRNA  
15 arrays. Deregulation of several miRNAs detected in this study may contribute to specific  
16 features of the disease; however, we are aware that their impact on the MDS characteristics  
17 has to be fully clarified by functional studies.

18

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24

1 **CONFLICT OF INTEREST**

2 The authors declare no conflict of interest.

3

4 **SUPPLEMENTARY MATERIAL**

5 Supplementary information is available at European Journal of Human Genetics's website.

6 **Supplementary Table 1.** Patients' karyotypes.

7 **Supplementary Table 2.** Normalized signal intensities of all 774 detected miRNAs  
8 (detection P-value < 0.01) in CD34+ cells of individual MDS/AML with MLD patients and  
9 healthy donors. Background signal was subtracted.

10

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1 **Table 1.** Patient characteristics.

<b>Patients</b>	<b>N</b>	<b>Sex (F/M)</b>	<b>Age (average, range)</b>	<b>Blasts, % (average, range)</b>	<b>WBC, 10<sup>9</sup>/l (average, range)</b>	<b>Karyotype (normal/abnormal /not available)</b>
<b>5q- syndrome</b>	6	6/0	66 (57-75)	2.3 (1.4-5)	4.6 (2.4-6.0)	0/6/0
<b>RA/RARS</b>	4/1	0/5	62 (57-76)	1.1 (0.2-4.2)	6.6 (4.6-9.0)	5/0/0
<b>RCMD</b>	6	0/6	66 (59-79)	2.7 (1.4-4.2)	5.5 (2.2-10.6)	3/0/3
<b>RAEB-1</b>	7	3/4	65 (58-72)	7.2 (3.6-9.2)	2.2 (1.7-33.1)	2/5/0
<b>RAEB-2</b>	10	4/6	60 (31-79)	14.0 (10.0-18.6)	3.2 (1.1-4.7)	1/9/0
<b>AML with MLD</b>	9	3/6	63 (47-73)	31 (20.4-56.2)	22.9 (0.5-154.4)	2/7/0

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1 **Table 2:** Previously published or putative miRNA targets with potential implications in MDS.

2

miRNA	miRNA deregulation in MDS	Previously published or putative targets
miR-10a	↑ early MDS ↓ advanced MDS	<i>HOX</i> genes (e.g. <i>HOXA1</i> , <i>HOXA3</i> , <i>HOXB3</i> ) <sup>33</sup>
miR-34a	↑ early MDS	<i>BCL2</i> <sup>36</sup> , <i>MEK1</i> <sup>40</sup> , <i>MET</i> <sup>41</sup> , <i>MYCN</i> , <i>SPII</i> *
miR-378	↓ in 5q- syndrome	<i>SUFU</i> * <sup>29</sup> , <i>ETV6</i> , <i>BCL2L2</i>
miR-127	↑ in all MDS	<i>BCL6</i> * <sup>21</sup> , <i>BLIMP1</i> <sup>20</sup> , <i>XBPI</i> <sup>20</sup>

3 \*Genes with inverse association of their transcript levels (compared with miRNA expression)

4 in our previously published mRNA expression data<sup>11</sup>.

5

1 **TITLES AND LEGENDS TO FIGURES**

2 **Figure 1:** Hierarchical clustering of 22 differentially expressed miRNAs between patients  
3 (MDS or AML with MLD) and controls ( $p < 0.001$ ). The binary logarithm of relative miRNA  
4 expression changes is expressed by a color gradient intensity scale, as shown at the top. Green  
5 color indicates down-regulation, red color indicates up-regulation of miRNA expression. Each  
6 column represents a separate sample and each row a single miRNA. N-control samples.

7

8 **Figure 2:** Hierarchical clustering of 45 differentially expressed miRNAs between different  
9 subtypes of the disease (5q- syndrome, low risk MDS (RA, RARS and RCMD), RAEB-1,  
10 RAEB-2 and AML with MLD) ( $p < 0.001$ ). The binary logarithm of relative miRNA  
11 expression changes is expressed by a color gradient intensity scale, as shown at the top. Green  
12 color indicates down-regulation, red color indicates up-regulation of miRNA expression. Each  
13 column represents a separate sample and each row a single miRNA. Particular miRNA  
14 clusters are color-coded on the right side of heatmap. N-control samples.

15

16 **Figure 3:** Differentially expressed miRNAs in patients with and without the deletion at 5q32  
17 region. The binary logarithm of relative miRNA expression changes is expressed by a color  
18 gradient intensity scale, as shown at the top. Green color indicates down-regulation, red color  
19 indicates up-regulation of miRNA expression. Each column represents a separate sample and  
20 each row a single miRNA. N-control samples.

21

22 **Figure 4:** Expression of miRNAs located within commonly deleted region at 5q32 (miR-143,  
23 miR-145, miR-378) or in its proximity (miR-146a) and their complementary variants (miR\*)  
24 measured by Illumina microarrays. Values are expressed as binary logarithms of fold changes  
25 (logFC). Boxes represent means and 25-75 percentiles and bars indicate the minimum and

1 maximum values for particular patient category compared to the control group (NORM). The  
2 5q- bar represents only patients with pure 5q- syndrome, whereas the bar denoted del(5q)  
3 illustrates miRNA expression in all patients with deletion at 5q32 region (all 5q- together with  
4 RAEB-1\_3, RAEB-2\_3, RAEB-2\_4, RAEB-2\_9, RAEB-2\_10 and AML with MLD\_6  
5 patients). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; other differences between normal samples and  
6 patients with del(5q) were not statistically significant. Expression of miR-145\* and miR-  
7 146a\* was under the detection limit of our experiment.

8

9 **Figure 5:** Validation of microarray data of selected miRNAs using qRT-PCR. Relative fold  
10 changes (FC) of gene expression were measured in the complete set of samples and were  
11 calculated by  $\Delta\Delta\text{CT}$  method. Values are expressed as binary logarithms of FC. Bars indicate  
12 the mean value of logFC plus standard error for particular patient category compared to the  
13 control group (NORM). The 5q- bar represents only patients with pure 5q- syndrome, whereas  
14 the bar denoted del(5q) illustrates miRNA expression in all patients with deletion at 5q32  
15 region (all 5q- together with RAEB-1\_3, RAEB-2\_3, RAEB-2\_4, RAEB-2\_9, RAEB-2\_10  
16 and AML with MLD\_6 patients).

-5,0

1:1

5,0

N1

N2

N3

N4

N5

N6

5q\_1

5q\_2

5q\_3

5q\_4

5q\_5

5q\_6

RA

RARS

RCMD\_1

RCMD\_2

RCMD\_3

RCMD\_4

RCMD\_5

RCMD\_6

RAEB-1\_1

RAEB-1\_2

RAEB-1\_3

RAEB-1\_4

RAEB-1\_5

RAEB-1\_6

RAEB-1\_7

RAEB-2\_1

RAEB-2\_2

RAEB-2\_3

RAEB-2\_4

RAEB-2\_5

RAEB-2\_6

RAEB-2\_7

RAEB-2\_8

RAEB-2\_9

RAEB-2\_10

AML with MLD\_1

AML with MLD\_2

AML with MLD\_3

AML with MLD\_4

AML with MLD\_5

AML with MLD\_6

AML with MLD\_7

AML with MLD\_8

hsa-miR-432

hsa-miR-323-3p

hsa-miR-299-5p

hsa-miR-409-3p

hsa-miR-329

hsa-miR-654-5p

HS\_40

HS\_206

hsa-miR-299-3p

hsa-miR-370

hsa-miR-494

hsa-miR-431

hsa-miR-665

hsa-miR-196 a\*

hsa-miR-507

hsa-miR-583

HS\_122.1

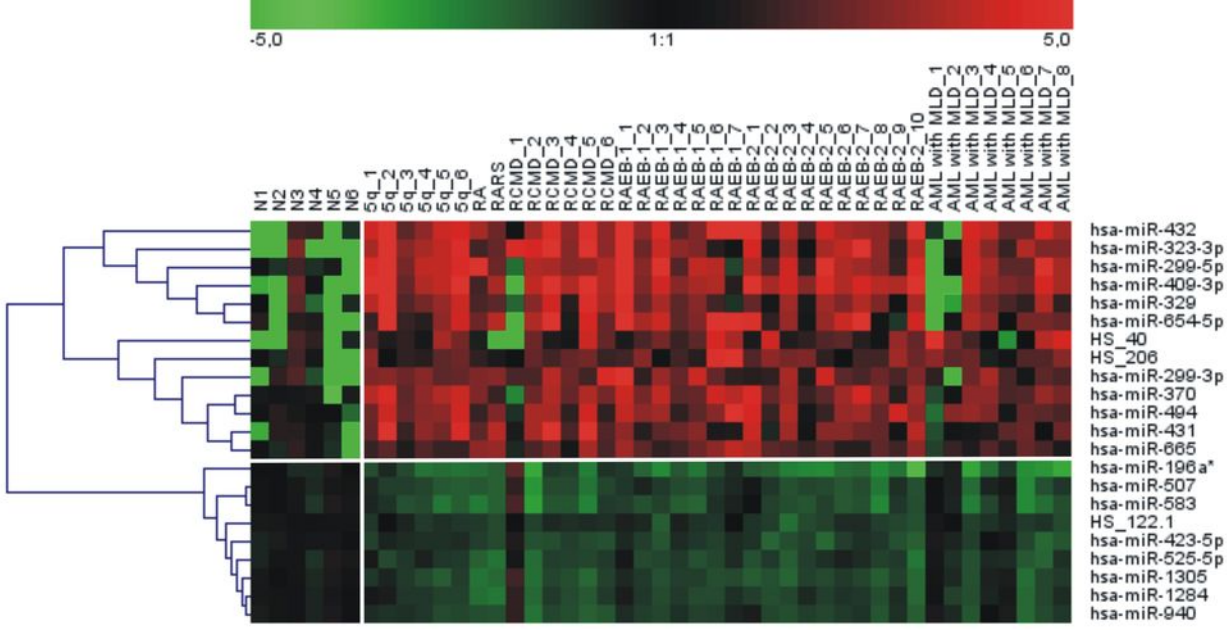
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hsa-miR-1305

hsa-miR-1284

hsa-miR-940



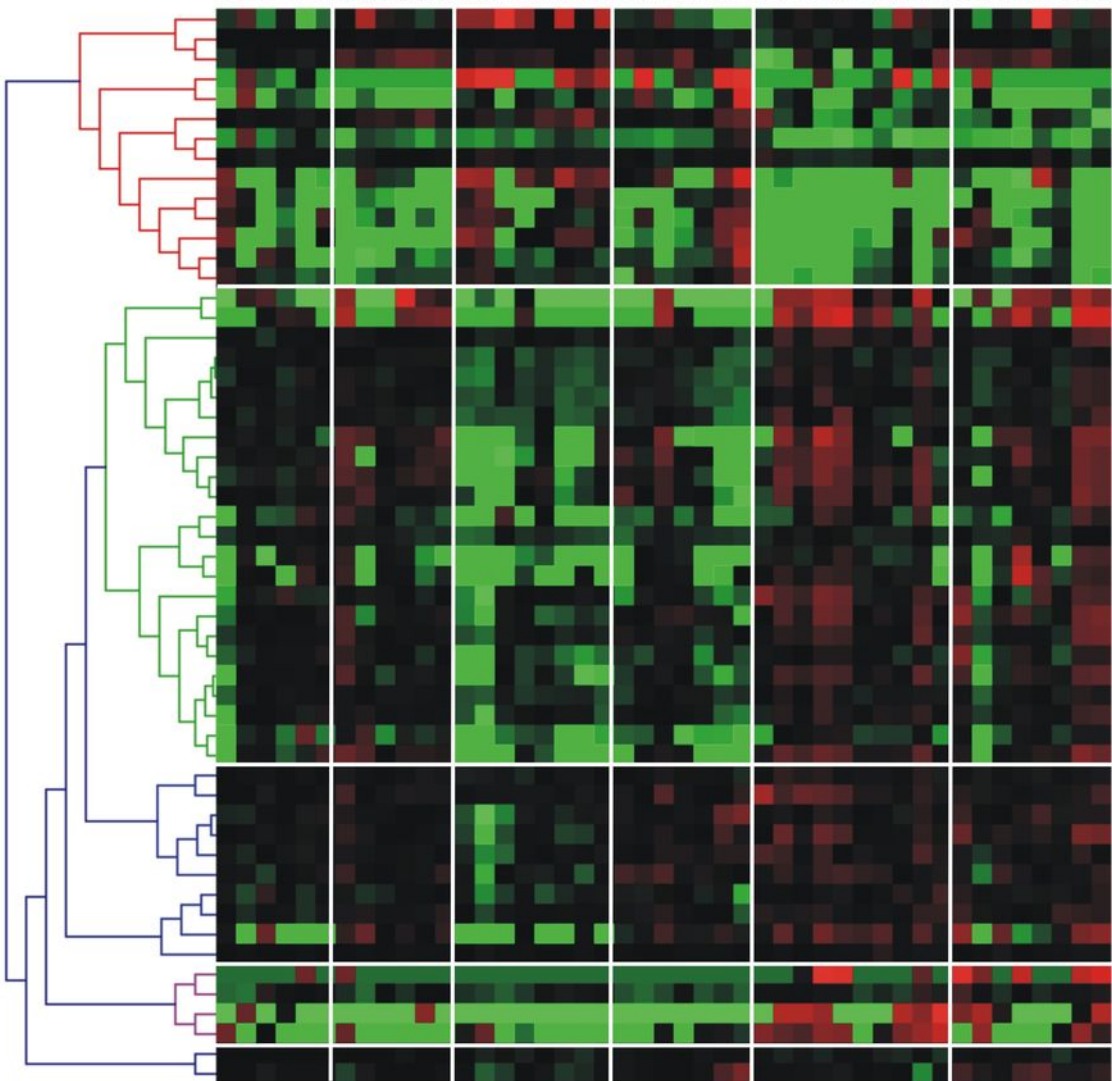


-5,0

1:1

5,0

N1 N2 N3 N4 N5 N6 5q\_1 5q\_2 5q\_3 5q\_4 5q\_5 5q\_6 RA RARS RCMD\_1 RCMD\_2 RCMD\_3 RCMD\_4 RCMD\_5 RCMD\_6 RAEB\_1 RAEB\_1\_2 RAEB\_1\_3 RAEB\_1\_4 RAEB\_1\_5 RAEB\_1\_6 RAEB\_1\_7 RAEB\_2\_1 RAEB\_2\_2 RAEB\_2\_3 RAEB\_2\_4 RAEB\_2\_5 RAEB\_2\_6 RAEB\_2\_7 RAEB\_2\_8 RAEB\_2\_9 RAEB\_2\_10 AML with MLD\_1 AML with MLD\_2 AML with MLD\_3 AML with MLD\_4 AML with MLD\_5 AML with MLD\_6 AML with MLD\_7 AML with MLD\_8



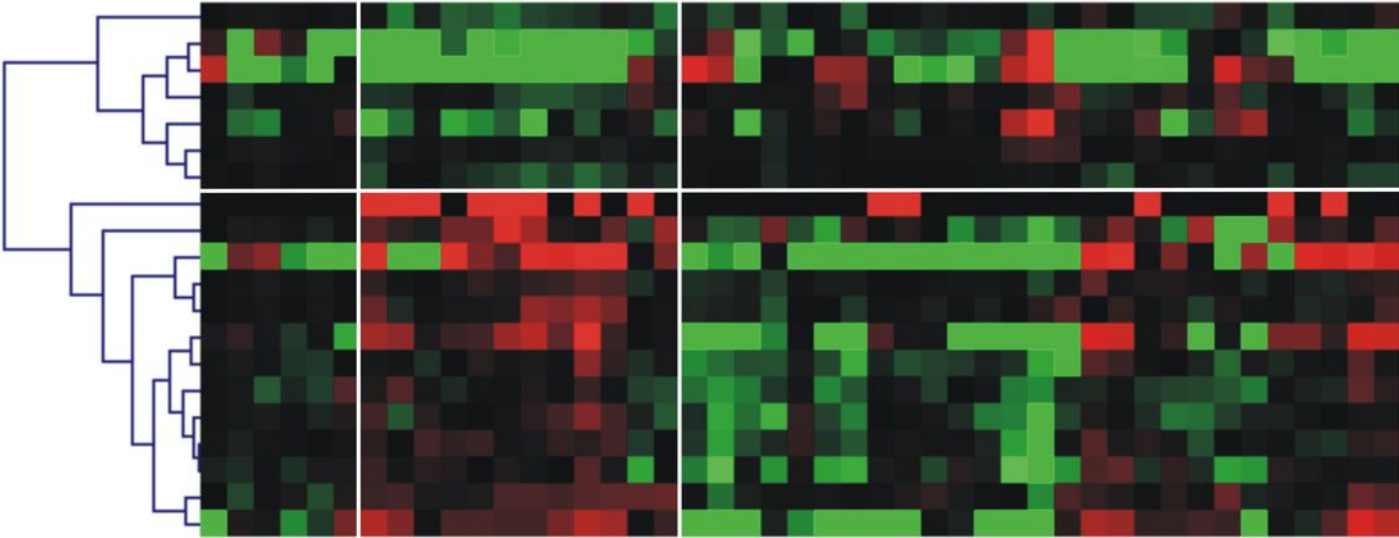
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- HS\_79.1
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- HS\_64
- HS\_278
- HS\_87
- HS\_3
- HS\_21
- hsa-miR-645
- hsa-miR-1183
- hsa-miR-138-2\*
- hsa-miR-30a
- HS\_20
- hsa-miR-1287
- hsa-miR-516a-3p
- hsa-miR-548a-3p
- hsa-miR-1288
- hsa-miR-624
- hsa-miR-146b-3p
- hsa-miR-922
- hsa-miR-1295
- hsa-miR-516a-5p
- hsa-miR-548m
- hsa-miR-625\*
- hsa-miR-212
- hsa-miR-30d\*
- HS\_113
- hsa-miR-371-5p
- hsa-miR-1206
- hsa-miR-372
- hsa-miR-875-5p
- hsa-miR-886-3p
- hsa-miR-936
- hsa-miR-103
- hsa-miR-326
- hsa-miR-298
- hsa-miR-548d-5p
- hsa-miR-671-3p
- hsa-miR-99b
- hsa-miR-505
- hsa-miR-148b
- HS\_59
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- HS\_114

-3.0

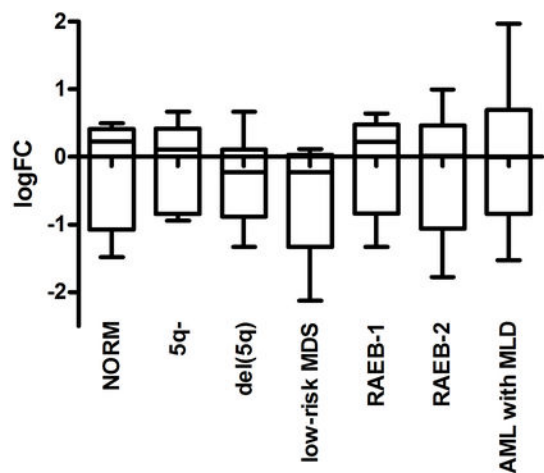
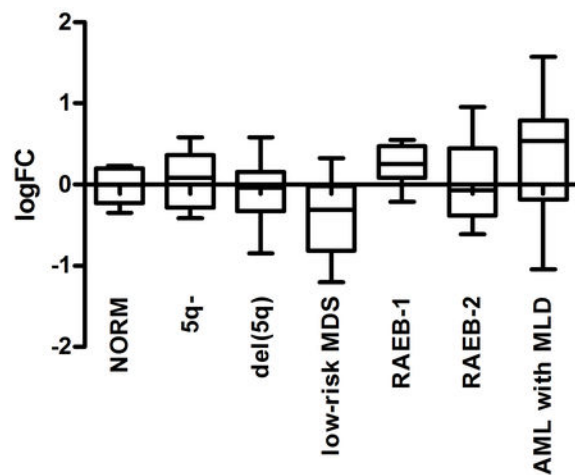
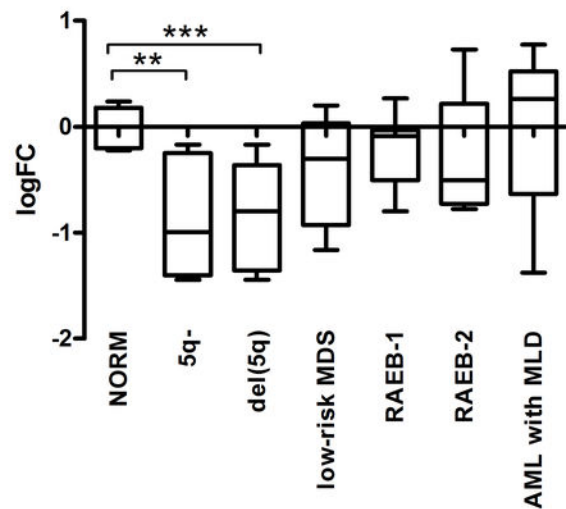
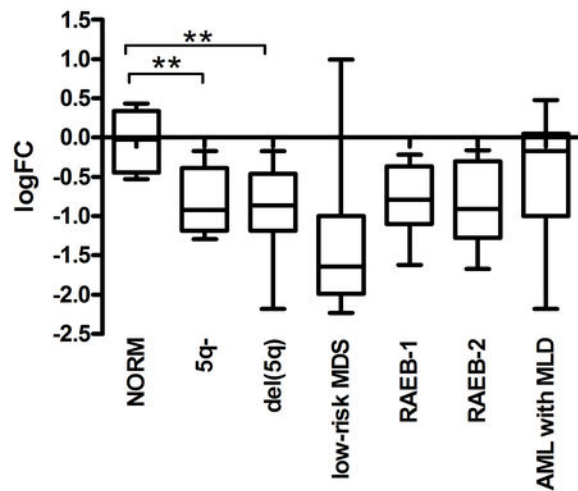
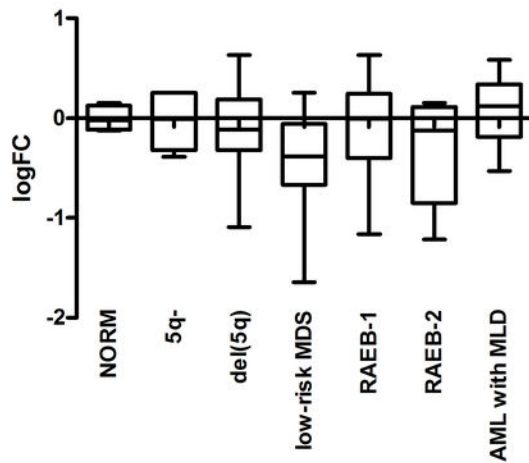
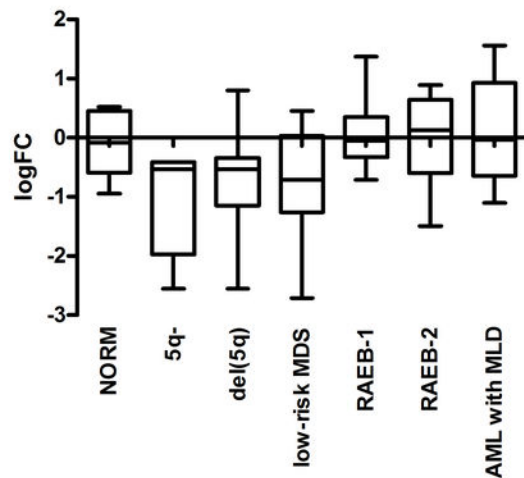
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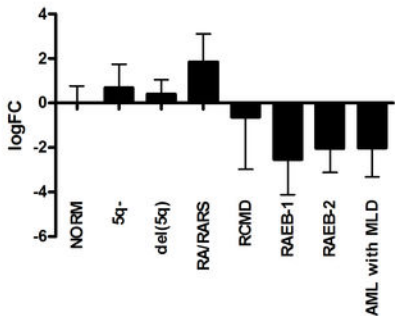
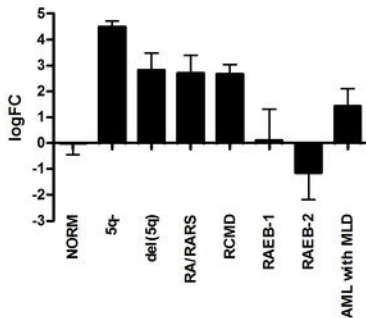
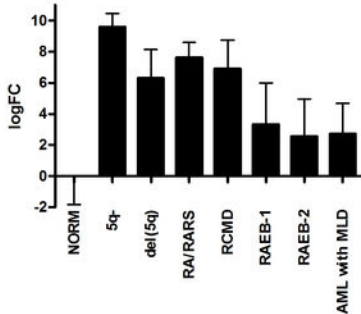
3.0

N1  
N2  
N3  
N4  
N5  
N6  
5q\_1  
5q\_2  
5q\_3  
5q\_4  
5q\_5  
5q\_6  
RAEB-1\_3\_5q  
RAEB-2\_3\_5q  
RAEB-2\_4\_5q  
RAEB-2\_9\_5q  
RAEB-2\_10\_5q  
AML with MLD\_6\_5q  
RA  
RARS  
RCMD\_1  
RCMD\_2  
RCMD\_3  
RCMD\_4  
RCMD\_5  
RCMD\_6  
RAEB-1\_1  
RAEB-1\_2  
RAEB-1\_4  
RAEB-1\_5  
RAEB-1\_6  
RAEB-1\_7  
RAEB-2\_1  
RAEB-2\_2  
RAEB-2\_5  
RAEB-2\_6  
RAEB-2\_7  
RAEB-2\_8  
AML with MLD\_1  
AML with MLD\_2  
AML with MLD\_3  
AML with MLD\_4  
AML with MLD\_5  
AML with MLD\_7  
AML with MLD\_8



hsa-miR-378  
HS\_21  
HS\_3  
hsa-miR-18a\*  
HS\_25  
hsa-let-7b  
hsa-miR-92a  
hsa-miR-556-5p  
hsa-miR-10a  
hsa-miR-1183  
hsa-miR-103  
hsa-miR-331-3p  
hsa-miR-1288  
hsa-miR-607  
hsa-miR-302c\*  
hsa-miR-107  
hsa-miR-340\*  
hsa-miR-651  
hsa-miR-148b  
hsa-miR-936

**miR-143****miR-145****miR-378****miR-143\*****miR-146a****miR-378\***

**miR-10a****miR-34a****miR-323-3p****miR-378**