Circulating microRNAs (miRNAs) are potential biomarkers for cancer. We examined plasma levels of 2 miRNAs, let-7a and miR-16, in 50 patients with myelodysplastic syndrome (MDS) and 76 healthy persons using quantitative real-time PCR. Circulating levels of both miRNAs were similar among healthy controls but were significantly lower in MDS patients ($P = .001$ and $P < .001$, respectively). The distributions of these 2 miRNA levels were bimodal in MDS patients, and these levels were significantly associated with their progression-free survival and overall survival (both $P < .001$ for let-7a; $P < .001$ and $P = .001$ for miR-16). This association persisted even after patients were stratified according to the International Prognostic Scoring System. Multivariate analysis revealed that let-7a level was a strong independent predictor for overall survival in this patient cohort. These findings suggest that let-7a and miR-16 plasma levels can serve as noninvasive prognostic markers in MDS patients. (Blood. 2011;118(2):413-415)

Methods

We retrospectively measured circulating levels of miRNAs let-7a and miR-16 in plasma samples from 50 randomly selected MDS patients who were seen at The University of Texas M. D. Anderson Cancer Center between 2004 and 2008 and from 76 healthy control individuals. The MDS patients had a median age of 73 years (range, 38-91 years) and a male-to-female ratio of 2:1. The MDS patient cohort represented the major pathologic groups defined in the 2008 World Health Organization classification of MDS and included the following: 25 patients with refractory cytopenia with multilineage dysplasia, 13 with refractory anemia with excess blasts (RAEB)-1, 9 with refractory anemia with excess blasts (RAEB)-2, 1 with MDS associated with isolated del(5q), 1 with refractory anemia with ring sideroblasts, and 1 with unclassified MDS (Table 1). These patients were also stratified according to their IPSS risk scores into 3 groups: low, intermediate-1, and intermediate-2. The 76 healthy control subjects were blood donors at The University of Texas M. D. Anderson Cancer Center. “Healthy” was defined as the absence of any type of infection or known medical condition at the time of study. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and the study was approved by the M. D. Anderson Cancer Center institutional review board.

miRNA levels were detected by quantitative real-time PCR with TaqMan miRNA assays (Applied Biosystems), with miR-192 as an internal control for plasma RNA normalization, as described previously. The relative expression level of each miRNA was calculated from the equation $2^{-\Delta C_t}$, where $\Delta C_t = \text{mean } C_t_{\text{miRNA}} - \text{mean } C_t_{\text{internal control}}$. Differences in miRNA levels were compared with the Student t test. Fisher exact and
2 tests were applied to categorical variables. The Kaplan-Meier method was used to generate overall survival (OS) and progression-free survival (PFS) curves. PFS was defined as time to progression to acute myeloid leukemia. Survival curves were compared with the log-rank test. To determine whether age, sex, morphology, IPSS score, or miRNA level were independent predictive factors for OS, we performed a multivariate analysis using the Cox proportional hazard model.

Results and discussion

We found that let-7a and miR-16 levels were stable in plasma of healthy control subjects. The relative mean level of let-7a was 23.78 ± 15.43 and that of miR-16 was 1140.01 ± 828.23. Levels of both miRNAs followed a gaussian distribution among the tested group (Figure 1A-B). Others have reported similar observations. Levels of both miRNAs were significantly lower in MDS patients (5.19 ± 37.51 for let-7a [P < .001] and 83.94 ± 337.77 for miR-16 [P < .001]), with each miRNA showing a bimodal distribution (Figure 1C-D). We therefore set an arbitrary cutoff value for each miRNA at the lowest frequency point between the 2 distribution peaks and divided the patients into high and low groups. The mean relative levels of let-7a were 28.97 ± 88.50 and 0.01 ± 0.02 in the high and low groups (P = .043), respectively, and those of miR-16 were 348.85 ± 719.81 and 9.24 ± 15.09 (P < .005), respectively.

IPSS score was significantly associated with OS (P = .022) in these MDS patients, but it was not significantly associated with PFS (P = .063; Figure 1E-F). We further plotted OS and PFS according to miRNA plasma levels. We found that miRNA levels predicted OS and PFS in the MDS patient group (Figure 1G-J; supplemental Table 1). Moreover, miRNA level could be used to further stratify patients in each IPSS category into different survival groups (supplemental Figure 1A-D). Similar results were obtained with a new risk model proposed by the M. D. Anderson Cancer Center (supplemental Figure 2A-H). On multivariate Cox analysis (supplemental Table 2), we found that an IPSS score of

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>let-7a</th>
<th>miR-16</th>
</tr>
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<tbody>
<tr>
<td>Low</td>
<td>High</td>
<td>P</td>
</tr>
<tr>
<td>≥ 72</td>
<td></td>
<td>.52</td>
</tr>
<tr>
<td>&lt; 72</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Sex</td>
<td>.18</td>
<td>.73</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>28 (74)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>10 (26)</td>
</tr>
<tr>
<td>WHO classification</td>
<td>.59</td>
<td>.38</td>
</tr>
<tr>
<td>MDS-U</td>
<td>0 (0)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>MDS del(5q)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>RCMD</td>
<td>19 (50)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>RARS</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>RAEB-1</td>
<td>10 (26)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>RAEB-2</td>
<td>7 (18)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Median survival, mo (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progression-free survival</td>
<td>(12-75)</td>
<td>12 (2-12)</td>
</tr>
<tr>
<td>Overall survival</td>
<td>42 (7-75)</td>
<td>8 (3-41)</td>
</tr>
</tbody>
</table>

Values are n (%) except for median survival. WHO indicates World Health Organization; MDS-U, MDS-unclassified; MDS del(5q), MDS associated with isolated del(5q); RCMD, refractory cytopenia with multilineage dysplasia; RARS, refractory anemia with ring sideroblasts; RAEB, refractory anemia with excess blasts; and INT, intermediate.
intermediate-2 and a high let-7a level were independent predictive factors for OS (hazard ratio 4.99, 95% confidence interval 1.60-15.59, \( P = .006 \), and hazard ratio 5.18, 95% confidence interval 1.62-16.60, \( P = .006 \), respectively). The levels of let-7a and miR-16 did not correlate significantly with cytopenia (\( P = .490 \) and \(.176 \), respectively) or karyotype (\( P = .425 \) and \(.467 \), respectively) in the present study cohort.

Let-7a is a tumor suppressor gene that regulates oncogenes such as RAS and HMGA2,\(^{20,21}\) and miR-16 targets multiple oncogenes, including BCL2, MCL1, CCND1, and WNT3A.\(^{23}\) Both of these miRNAs are down-regulated in chronic lymphocytic leukemia, pituitary adenomas, and prostate carcinoma.\(^{15,16,22}\) Decreased MiR-16 expression also has been found in blasts isolated from high-risk MDS patients.\(^{23}\) The exact mechanisms by which circulating miRNAs regulate certain biologic functions are unknown. Previous findings have suggested that miRNAs function as “extracellular communication RNAs” that play an important role in cell proliferation and differentiation.\(^{24,25}\) If true, the findings we report suggest that antiproliferative and proapoptotic miRNA activities are down-regulated in the extracellular environment during the phase of MDS when cells in the bone marrow undergo massive apoptosis. These activities, however, are up-regulated when MDS progresses into a proliferative phase.

This is the first report in which plasma miRNA levels in MDS patients have been assessed. We found that miR-16 and let-7a levels were significantly different between healthy control subjects and MDS patients, which makes them possible early, noninvasive biomarkers for diagnosis or prognosis of MDS patients. If confirmed by other studies, assessment of plasma levels of let-7a and miR-16 miRNA may add to the current IPSS risk model for predicting MDS patient survival. Our findings also suggest that extracellular miRNAs play important roles in the development and progression of MDS.

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Authorship

Contribution: Z.Z. and G.A.C. performed research, analyzed data, and wrote the manuscript; H.M.d.P. collected clinical data; L.J.M. analyzed data and helped to write the manuscript; M.H.F. and M.S. performed experiments; G.G.-M. evaluated clinical characteristics and provided samples; and C.E.B.-R. designed the research, analyzed the data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

Circulating microRNAs let-7a and miR-16 predict progression-free survival and overall survival in patients with myelodysplastic syndrome

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