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

Short Title: Circulating microRNAs predict survival in MDS

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Scientific Category: Myeloid Neoplasia
Abstract

Circulating microRNAs (miRNAs) are potential biomarkers for cancer. We examined plasma levels of two 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 two 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, and $P < .001$ 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.
Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders characterized by abnormalities of bone marrow hematopoietic cells and the microenvironment. MDS patients have a variable risk of transformation to acute myeloid leukemia (AML) and transformation is believed to be a multi-step process requiring the accumulation of genetic and epigenetic alterations. Alterations in apoptosis and proliferation have been implicated in the pathogenesis of MDS, but the mechanisms underlying these alterations are incompletely understood. The widely used risk model, the International Prognostic Scoring System (IPSS), integrates cytogenetics, morphology and clinical features, but is limited in its ability to predict MDS patient outcomes. Molecular markers are needed to improve prediction accuracy.

MicroRNAs (miRNAs) are a recently discovered class of short (19-25 nt), naturally occurring, single-stranded RNA molecules that are components of the epigenetic machinery. MiRNAs regulate the expression of target genes post-transcriptionally, mostly by inhibiting translation or inducing mRNA degradation. MiRNAs also play important roles in the regulation of DNA methylation and histone modification and can function as oncogenes, tumor suppressor genes, or both. MiRNAs have been specifically implicated in the development of solid and hematopoietic malignancies. Recently, miRNAs were identified in several types of body fluid, from both healthy individuals and patients with various types of cancer, and may therefore have potential as noninvasive biomarkers of cancer. One recent study found that plasma levels of miR-92 may be a biomarker for AML.

Two miRNAs, let-7a and miR-16, are known to play important roles in myeloid leukemogenesis by regulating the cell cycle and apoptosis, both of which are important in MDS pathogenesis. We decided to focus on these two miRNAs that are known to be downregulated in leukemias. The goal of this study was to analyze the levels of let-7a and miR-16 in plasma samples from MDS patients to assess their potential clinical significance.
Methods

We retrospectively measured circulating miRNAs let-7a and miR-16 levels in plasma samples from 50 randomly selected MDS patients who were seen at The University of Texas MD Anderson Cancer Center (UTMDACC) 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 WHO classification of MDS\(^4\) and included: 25 refractory cytopenia with multilineage dysplasia, 13 refractory anemia with excess blasts (RAEB)-1, 9 RAEB-2, 1 MDS associated with isolated del(5q), 1 refractory anemia with ring sideroblasts, and 1 MDS-unclassified. These patients were also stratified according to their IPSS risk scores into three groups: low, intermediate 1 (INT-1), and intermediate 2 (INT-2). The 76 healthy controls were blood donors at UTMDACC. “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 polymerase chain reaction (qRT-PCR) using TaqMan MicroRNA Assays (Applied Biosystems, Foster City, CA), with miR-192 as an internal control for plasma RNA normalization, as described previously.\(^{19}\) The relative expression level of each miRNA was calculated from the equation \(2^{-\Delta Ct}\), where \(\Delta Ct = mean Ct_{miRNA} - mean Ct_{internal\ control}\). Differences in miRNA levels were compared using the Student’s \(t\)-test. Fisher’s exact and Chi-square 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 AML. Survival curves were compared using the log-rank test. To determine if 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 controls. The relative mean level of let-7a was 23.78±15.43 and that of miR-16, 1140.01±828.23. Levels of both miRNAs followed a Gaussian distribution among the tested group (Figure 1A-B). Others have reported similar observations.\(^{10}\) 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 \(P < .001\)), 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 two distribution peaks, and divided the patients into high (H) and low (L) groups. The mean relative levels of let-7a were 28.97±88.50 and 0.01±0.02 in the H and L groups \(P = .043\), respectively, and those of miR-16 were 348.85±719.81 and 9.24±15.09 in the H and L groups \(P = .005\), respectively.

IPSS score was significantly associated with OS \(P = .022\) in these MDS patients, but not significantly associated with PFS \(P = .063\) (Figure 1E and F). We further plotted OS and PSF according to miRNA plasma levels. We found that miRNA levels predicted OS and PFS in the MDS patient group (Figure 1G-J and Table S1). Moreover, miRNA level could be used to further stratify patients in each IPSS category into different survival groups (Figure S1A-D). Similar results were obtained using a new risk model proposed by the MD Anderson Cancer Center (Figure S2A-H). On multivariate Cox analysis (Table S2), we found that an IPSS score of INT-2 and a high let-7a level were independent predictive factors for OS (HR = 4.99, 95% CI = 1.60-15.59, \(P = .006\), and HR = 5.18, 95% CI = 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 this study cohort.
Let-7a is a tumor suppressor gene that regulates oncogenes such as RAS and HMGA2, and miR-16 targets multiple oncogenes, including BCL2, MCL1, CCND1, and WNT3A. Both of these miRNAs are downregulated in chronic lymphocytic leukemia, pituitary adenomas, and prostate carcinoma. Decreased MiR-16 expression also has been found in blasts isolated from high-risk MDS patients. The exact mechanisms by which circulating miRNAs regulate certain biological functions are unknown. Previous findings have suggested that miRNAs function as “extracellular communication RNAs” that play an important role in cell proliferation and differentiation. If true, the findings we report suggest that antiproliferative and proapoptotic miRNA activities are downregulated in the extracellular environment during the phase of MDS when cells in the bone marrow undergo massive apoptosis. These activities, however, are upregulated 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 controls and MDS patients, making 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.

Acknowledgments

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Authorship contributions

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

Disclosure of conflicts of interest

The authors declare no competing financial interests.

References


Table 1. Clinicopathologic characteristics and survival of MDS patients according to circulating let-7a and miR-16 levels

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>let-7a</th>
<th>miR-16</th>
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<tbody>
<tr>
<td></td>
<td>Low no. (%)</td>
<td>High no. (%)</td>
<td>P</td>
<td>Low no. (%)</td>
</tr>
<tr>
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<td>≥72</td>
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<td>&lt;72</td>
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<td>6 (50)</td>
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<td>16 (41)</td>
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<tr>
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<td>.73</td>
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<td>26 (67)</td>
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<td>13 (33)</td>
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<tr>
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<td>RCMD</td>
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<td>INT-2</td>
<td>16 (42)</td>
<td>5 (42)</td>
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<td>15 (38)</td>
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<td>Median survival, mo (range)</td>
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<td>Progression-free survival</td>
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<td>- (13-75)</td>
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<td>Overall survival</td>
<td>42 (7-75)</td>
<td>8 (3-41)</td>
<td>&lt;.001</td>
<td>42 (5-75)</td>
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</table>

MDS indicates myelodysplastic syndrome; MDS-U indicates myelodysplastic syndrome-unclassified; MDS del(5q) indicates MDS associated with isolated del(5q); RCMD indicates refractory cytopenia with multilineage dysplasia; RARS indicates refractory anemia with ring sideroblasts; RAEB indicates refractory anemia with excess blasts; INT, indicates intermediate.
Figure legend

Figure 1. Distributions of let-7a and miR-16 plasma levels in 76 healthy controls individuals (A and B) and 50 MDS patients (C and D), and Kaplan-Meier curves showing (E) OS by IPSS risk score (P = .022); (F) PFS by IPSS risk score (P = .063); (G) OS by let-7a level (P < .001); (H) PFS by let-7a level (P < .001); (I) OS by miR-16 level (P = .001). (J) PFS by miR-16 level (P < .001). OS, overall survival; PFS, progression-free survival; IPSS, International Prognostic Scoring System; INT, intermediate; MDS, myelodysplastic syndrome.
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