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Implications of the miR-10 family in chemotherapy response of NPM1-mutated AML

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Key Points

- High miR-10 family expression levels in AML patients are associated with achieving complete remission to induction chemotherapy.
- Functional experiments did not show any impact of miR-10a-5p in AML blast growth or survival at baseline conditions or after chemotherapy.

Introduction

Mutations of the nucleophosmin (NPM1) gene, usually occurring at exon 12, represent the most common genetic alteration in cytogenetically normal acute myeloid leukemia (CN-AML) (50% to 60% of cases) and account for about one-third of all adult AML.1-3 The presence of NPM1 mutations is associated with complete remission (CR) rates in young and elderly AML patients (70% to 85%).1,2 However, the mechanisms responsible for such an effect on chemotherapy response are unknown. Unraveling those mechanisms will be critical to understand why NPM1 mutations are associated with high CR and may provide novel targets for therapeutic development.

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression by repressing or promoting translation and/or accelerating messenger RNA decay.4 MiRNA expression is dysregulated in AML blasts5 and is associated with specific cytogenetic and molecular alterations.5-8 It has been shown that a unique miRNA signature is associated with CN-AML patients harboring NPM1 mutations and includes the strong upregulation of miR-10a-5p and miR-10h-5p.7,8 However, the functional role of these 2 miRNAs in NPM1-mutated AML (NPM1mut-AML) is unknown. Here, we asked whether the miR-10 family could predict chemotherapy response in AML and whether the chemotherapy sensitivity and high CR rates observed in NPM1mut-AML patients could be mediated through miR-10 family members. To answer these questions, we analyzed miR-10 family expression in relation to CR and NPM1 mutation status in 2 cohorts of de novo AML patients. Furthermore, we performed miR-10a-5p gain- and loss-of-function studies using AML cell lines and primary AML samples before treatment and after chemotherapy.

Study design

Patient samples and microarray experiments

Pretreatment miRNA expression was analyzed in 54 de novo adult AML patients obtained from the MD Anderson Cancer Center Leukemia Tissue Bank using the Ohio State University (OSU) miRNA microarray (version 3).5 MiR-10 family expression levels were also measured in a second cohort of 183 older (age ≥ 60 years) de novo CN-AML patients treated on CALGB/ALLIANCE protocols using the OSU miRNA microarray (Gene Expression Omnibus accession number GSE55519). Patient characteristics are shown in supplemental Tables 1 and 2. These studies were performed under the OSU institutional review board protocol 2005CO0014. These studies were conducted in accordance with the Declaration of Helsinki.

Cell transfections and functional experiments

See figure legends and supplemental Methods (available on the Blood Web site) for details.

The online version of this article contains a data supplement.


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Figure 1. miR-10 family expression in relation to in vitro and in vivo chemotherapy response in AML. (A) Baseline miR-10a-5p expression in untreated AML samples according to chemotherapy response (no-CR vs CR). (B) Baseline miR-10a-5p expression in untreated AML samples according to chemotherapy response (no-CR vs CR). Measures of expression were obtained after normalization and background subtraction of miRNA microarray data from 54 newly diagnosed AML patients. (C) Baseline miR-10a-5p levels in AML cell lines detected by quantitative real-time polymerase chain reaction. Results are shown after normalization with U44. (D) Cell growth curves of KG-1a and Kasumi-1 cells (left panel) or K562 and OCI-AML3 cells (right panel) transfected/infected in vitro with 100 nM of synthetic miR-10a-5p, miR-10a-5p overexpressing lentivirus (pMIR-10a), or anti-miR-10a-5p (anti-10a-5p) oligonucleotides and their respective controls (scrambled oligonucleotides or empty vector [pMIR-EV]). The rationale for using a lentivirus expressing miR-10a-5p to infect Kasumi-1 cells was based on the difficulty in achieving successful overexpression of synthetic miR-10a-5p using nucleoporation methods. The efficiency of infection of the Kasumi-1 cell line is 50% using a multiplicity of infection of 5. The efficiency of nucleoporation of the KG-1a cell line is about 80% to 90%.

Cells were harvested and counted at 24-hour intervals using a ViCell counter (Beckman Coulter). Each sample was run in triplicate. (E) Annexin V/propidium iodide (PI) assays in KG-1a and Kasumi-1 (left panel) and K562 and OCI-AML3 (right panel) cells after 48 hours of transfection/infection with synthetic miR-10a-5p/lentivirus pMIR-10a, anti–miR-10a-5p, or controls (scrambled oligonucleotides or empty vector [pMIR-EV]). Experiments were repeated in triplicate. (F) Annexin V/PI assays in 2 primary CN-AML patient samples with NPM1-FLT3 wild-type and low level of miR-10a-5p. About 3 x 10^6 cells were cultured with StemSpan SFEM supplemented with 20% fetal bovine serum and StemSpan CC100 cytokine cocktail (STEMCELL Technologies) along with transferrin-conjugated nanoparticles (NPs) encapsulated with 0.4 μM synthetic miR-10a-5p or scrambled controls. nanoparticle-conjugated miRNAs was used to improve cell delivery as described in detail. In addition, ARA-C (5 μM) or control (PBS) was added to the culture media. The miR-10a-5p expression in primary AML blasts after nanoparticle-conjugated synthetic miRNA coculture was assessed by quantitative real-time polymerase chain reaction (supplemental Figure 2). (G) Annexin V/PI assays in 2 primary CN-AML patient samples with FLT3 wild-type and NPM1-mutated high level of miR-10a-5p. About 3 x 10^6 cells were cultured with ARA-C (5 μM) or control (PBS) media as described in (F) along with transferrin-conjugated NPs encapsulated with 0.4 μM synthetic anti–miR-10a-5p or scrambled controls (see supplemental Figure 2 for miR-10-5p expression after nanoparticle-miRNA treatment).
Results and discussion

To identify whether miR-10 family members are associated with CR, we first analyzed the expression of these miRNAs in 54 de novo adult AML BM samples (supplemental Table 1) before treatment with idarubicin and cytarabine using the OSU miRNA microarray platform. Patients with induction deaths were excluded. First, we compared pretreatment miR-10a-5p and miR-10b-5p expression in patients who achieved CR (n = 28) vs those who did not (no-CR) (n = 26). The median miR-10a-5p levels were 12.1 (range, 4.2-14.2) in CR patients and 9.1 (range, 5.1-13.5) in no-CR patients (P = .003), whereas the median miR-10b-5p levels were 12.2 (range, 4.2-13.7) in CR patients and 8.1 (range, 4.2-13.1) in no-CR patients (P = .002) (Figure 1A-B and supplemental Table 3).9,10 Similar results were obtained when we compared pretreatment whole miRNA expression between CR and no-CR patients. We found a miRNA signature comprised of 9 miRNAs (>2 fold change, P < .05; supplemental Table 4), where the top 3 upregulated miRNAs in CR patients were miR-10a-3p, miR-10b-5p, and miR-10a-5p (fold change 8.3, 6.9, and 3.7, respectively).

Univariable logistic regression analyses for CR showed significant associations with cytogenetics, age, NPM1 status, and miR-10a-5p and miR-10b-5p (Table 1). We next assessed significance of miR-10 expression for CR when adjusting for these other significant factors (age, NPM1 status, unfavorable cytogenetic prognostic group, and CN-AML). In multivariable analysis incorporating all of the covariates in the model, we found that miR-10a-5p and NPM1 mutation status maintained significant associations with achievement of CR (P = .019 and P = .005, respectively; Table 1). Interestingly, when we included only miR-10a-5p and NPM1 mutation status in the model, NPM1 mutation status remained significant along with a significant interaction effect with miR-10a-5p, where NPM1 mutation status modifies the effect of miR-10a-5p on CR incidence (P = .033) (supplemental Figure 1). In the additional data set of older (age ≥ 60 years) de novo CN-AML patients treated on CALGB/ALLIANCE protocols, we also saw that miR-10a-5p and miR-10b-5p associated with CR status (P = .014 and P = .054, respectively) (supplemental Table 5). The univariable logistic regression significantly associated with NPM1 status, but not with miR-10a-5p or miR-10b-5p (supplemental Table 6). There was, however, a significant interaction between miR-10a-5p and NPM1 status (interaction P = .027). In the multivariable analyses after adjusting for BAALC expression status, we found the interaction between miR-10a-5p and NPM1 mutation status remained significantly associated with achievement of CR (interaction P = .010), whereas miR-10b-5p did not (P = .92) (Table 1).

Next, we asked whether the chemotherapy sensitivity and high CR rates observed in AML patients with NPM1 mutations is mediated through miR-10a-5p expression. To address this question, we first overexpressed miR-10a-5p in AML cell lines with low endogenous miR-10a-5p expression (KG-1a and Kasumi-1) (Figure 1C and supplemental Figure 2) and measured cell proliferation and apoptosis after treatment with cytarabine or controls. As shown in Figure 1D-E (left panels), no changes in cell proliferation or apoptosis between miR-10a-5p and the controls were observed. To investigate whether miR-10a-5p overexpression leads to granulocytic differentiation, the granulocytic marker CD11b and cell morphology were assessed after miR-10a-5p overexpression in Kasumi-1 cells. No differences in CD11b or cell morphology were observed up to 72 hours (not shown). Furthermore, we knocked down miR-10a-5p in 2 AML cell lines with high levels of miR-10a-5p (K562 and the NPM1-mutated OCI-AML311; see Figure 1C and supplemental Figure 2) using anti-miR-10a-5p or scrambled control. Subsequently, the cells were cultured in the presence or absence of cytarabine, and cell proliferation and apoptosis were measured. No differences in cell proliferation and apoptosis were observed for either cell line (Figure 1D-E, right panels). These results were confirmed in 4 primary AML patient samples: 2 with NPM1 wild-type and low levels of miR-10a-5p that were transfected with nanoparticle-conjugated synthetic miR-10a-5p, and 2 with NPM1-mutated and high levels of miR-10a-5p that were transfected with nanoparticle-conjugated antimiR-10a-5p. No significant difference was found in apoptosis after 48 hours with or without cytarabine (Figure 1F-G and supplemental Figure 2).

In this Brief Report, we demonstrated that high miR-10a-5p expression levels in AML patients are associated with achieving CR, but the interaction effects between NPM1 mutation status and miR-10a-5p expression was most significant overall and suggests that NPM1 mutation status modifies the effects of miR-10a-5p expression. We and others initially described that high expression of the miR-10 family strongly associated with chemotherapy sensitivity. However, the mechanism by which the presence of NPM1 mutations is associated with chemotherapy sensitivity and high CR rate is not known. Because miRNAs have been involved in chemotherapy resistance,13–15 we reasoned that the high CR rates observed in NPM1mut-AML patients could be mediated by the upregulation of miR-10a-5p expression and subsequent modulation of critical targets involved in chemotherapy sensitivity. However, our miR-10a-5p gain- and loss-of-function experiments in cell lines and in primary AML samples did not demonstrate any effect in apoptosis and cell proliferation in baseline conditions nor after cytarabine treatment. Thus, it is unlikely
that miR-10a-5p has any role in chemotherapy sensitivity of AML samples with NPM1 mutations. These experiments also argue against any critical role of this miRNA in leukemogenesis of NPM1mut-AML, because its inhibition in AML cell lines and primary samples does not result in any significant phenotype.

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Authorship

Contribution: V.H., P.R., C.E.A., C.M.C., D.N., S.M.K., M.A., G.M., C.D.B., and R.G. designed research; S.M.K, and C.D.B. provided patient samples and C.D.B. and M.A. provided clinical data; V.H., P.R., C.E.A., and D.N performed research; V.H., P.R., C.E.A., S.V., C.M.C., D.N., and R.G. analyzed data; and V.H. and R.G. wrote the paper. All authors critically reviewed and edited the paper.

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