Aneta Wiśnik

Analysis of selected microRNAs expression involved in the regulation of hematopoietic niche genes expression in patients with acute myeloid leukemia.

Aneta Wiśnik, Magdalena Czemerska, Agnieszka Wierzbowska, Izabela Zawlik
Hematology Department in Medical University of Lodz, Copernicus Memorial Hospital
E-mail: aneta.wisnik@stud.umed.lodz.pl

INTRODUCTION

MicroRNAs (miRNAs) are small, endogenous, single-stranded, around 19-25 nucleotides long, functional molecules representing an enormous class of non-coding RNAs. MiRNAs are highly evolutionarily conserved molecules, and nowadays, their role is still discovering. They are short regulatory RNAs which mediate posttranscriptional gene silencing, usually by complementary binding with 3' untranslated region (UTR) sequences in their target mRNA. A single miRNA can affect the expression of hundreds of messenger RNAs (mRNAs), resulting in their degradation or repression of translation. MiRNAs are responsible for many biological process regulations, and their expression level alterations may play an essential role in the multiple diseases' development. Altered miRNA expression level appears to be a well-known mechanism in **acute myeloid leukaemia** (**AML)**. Understanding the role of these short molecules is crucial in diagnosis, prognosis and therapy development for patients with AML. Searching for altered miRNAs expressed in AML could lead to better evaluation of prognosis for patients. Acute myeloid leukaemia is a malignant clonal disorder of hematopoietic stem and progenitor cells of bone marrow, which leads to excessive proliferation and accumulation of immature blast cells deriving from precursor, neoplastically transformed myeloid cells. The expression level of selected microRNAs can affect the biological profile of AML cells and perhaps influence patients' prognosis. The role of microRNA in bone marrow hematopoietic niche and its influence on hematopoietic stem cells remains unclear.

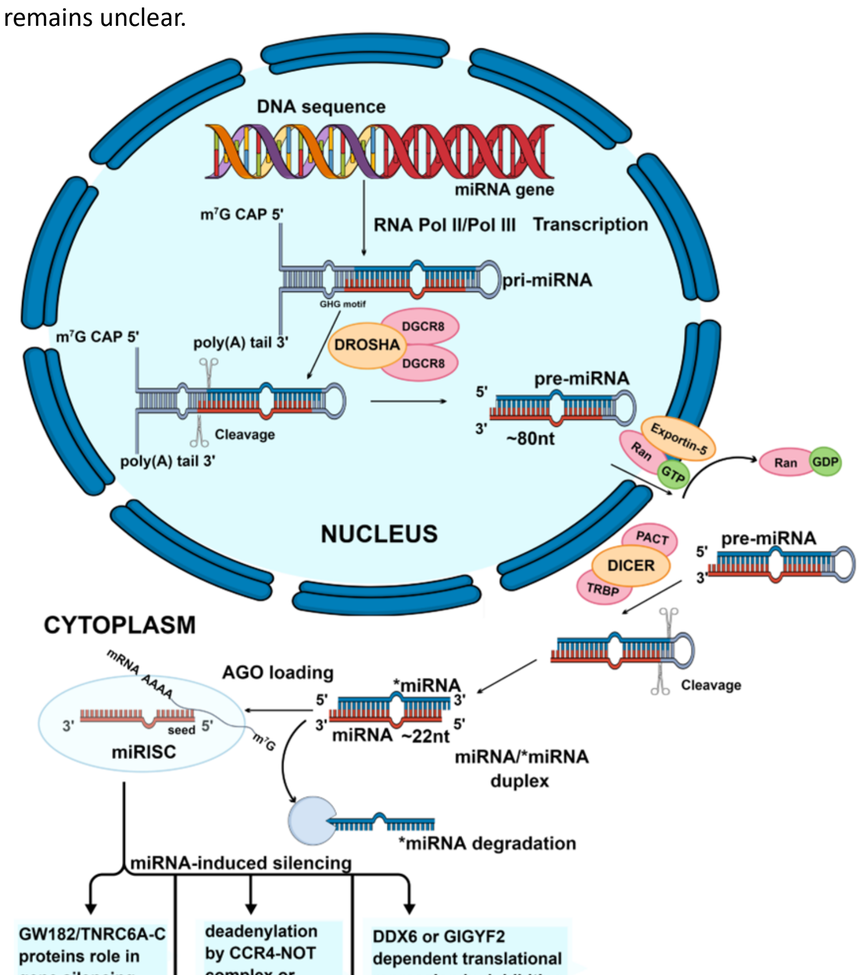

Figure 1. Scheme of microRNA synthesis and role on mRNA.

AIMS AND HYPOTHESIS

- **The study aims** to analyze the expression level of particular microRNAs present in scientific articles within the context of potential meaning in bone marrow hematopoietic niche and influence on hematopoietic stem cells.
- **The analysis aims** to compare the expression level of selected microRNAs with clinical parameters dependent on the patients (age of onset, gender) and dependent on the disease (molecular disorders, cytogenetic and molecular risk, the antigen expression on the surface of leukaemic cells, response to treatment, overall survival and event-free survival) in patients with AML.
- **The expression level of selected microRNAs can affect the biological profile of AML cells and perhaps influence patients' prognosis.**

SUBJECT OF RESEARCH

The subject of this study is an analysis of the expression level of 12 selected microRNAs: **has-miR-15a-5p, hsa-miR-34a-5p, hsa-miR-125b-5p, hsa-miR-139-5p, hsa-miR-146-5p, hsa-miR-181a-5p, hsa-miR-199b-5p, hsa-miR-204-5p, hsa-miR-218-5p,hsa-miR-299-5p, hsa-miR-424-3p, hsa-miR-452-5p** present in scientific articles within the context of potential meaning in bone marrow hematopoietic niche and influence on hematopoietic stem cells using of Real-Time PCR method in patients with AML.

METHODS

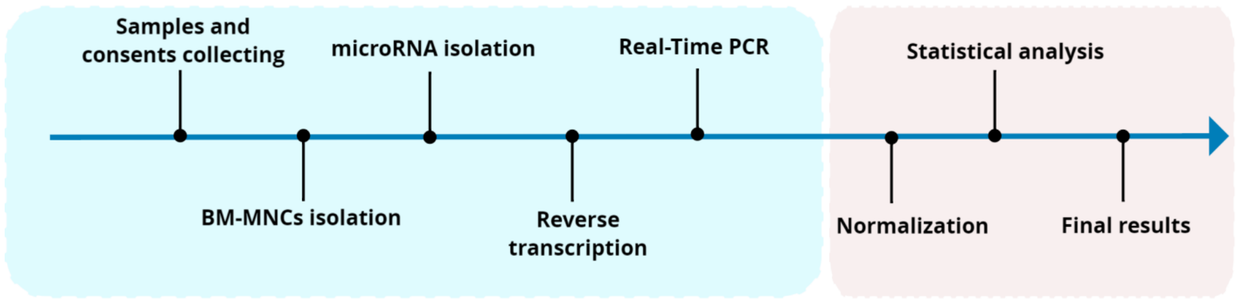

Figure 2. Methodology workflow and timeline.

MATERIALS

In this study, so far, **69 samples of bone marrow** have been collected from patients diagnosed with AML. Inclusive criteria for the study were AML diagnosis, bone marrow sample collected at the moment of diagnosis, absence of hemolysis in the sample, written consent from a patient for the examination and a sufficient number of MNCs. Exclusive criteria were insufficient MNCs from bone marrow, another diagnosis, e.g., acute promyelocytic leukaemia (APL), acute lymphoblastic leukaemia (ALL), and a sample collected at other stages of treatment. Within archived material, 69 samples were chosen for the study and were eligible for the inclusive criteria. Number of Bioethical Consent: **RNN/115/23/KE** dated 16th May 2023.

RESULTS

The expression analysis of 12 selected microRNAs, 3 endogenous controls and one spike-in control was conducted in the study group of 69 AML patients. The study group was characterized by gender, the age of onset, karyotype, molecular disorders, cytogenetical and molecular risk classification of EuropeanLeukemia Net 2022 (Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood 2022; 140 (12): 1345–1377)*.

Tabel 1. The study group characterization at the moment of AML diagnosis.

Median age	58 years
Number of women	n=32
Number of men	n=37
Number of patients with FLT3-ITD mutation	n=14
Number of patients with FLT3-TKD mutation	n=5
Number of patients with NPM1 mutation	n=14
Karyotype	favourable n=5 intermediate n=37 adverse n=13 no data n=14
Cytogenetic and molecular risk *ELN2022**	favourable n=11 intermediate n=7 adverse n=22 no data n=29

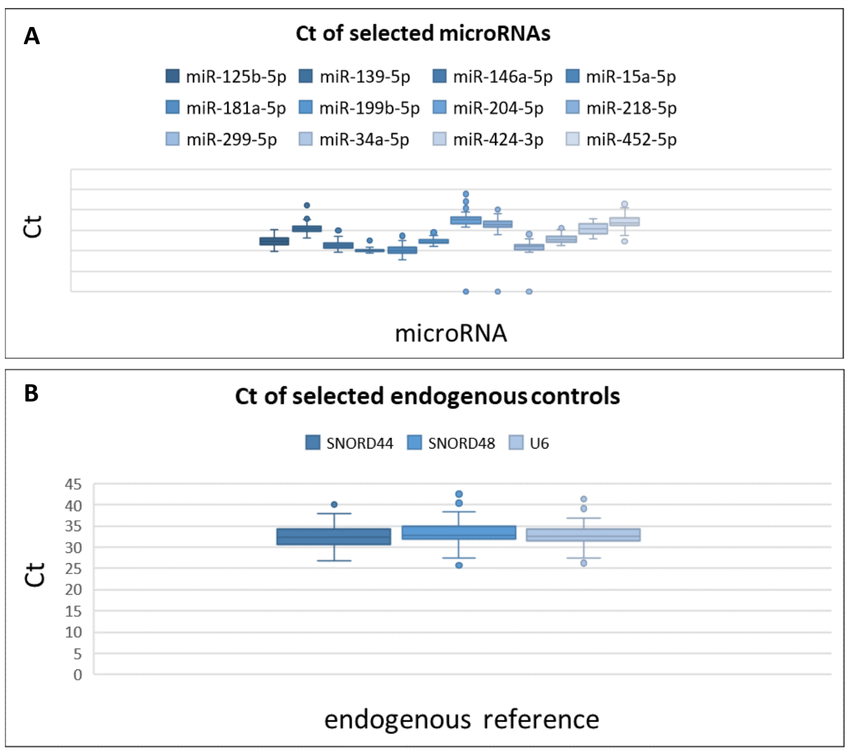

Figure 3. Ct data of selected microRNAs analyzed by Real-Time PCR (A). Ct data of selected endogenous controls analyzed by Real-Time PCR (B). Distribution of *FLT3-ITD* mutation in study group (C). Distribution of *FLT3-TKD* mutation in study group (D). Distribution of *NPM1* mutation in study group (E).