The role of sirtuin family histone deacetylases as biomarkers, predictive factors and potential therapeutic targets in patients with acute myeloid leukemia

International Doctoral School
Medical University of Lodz

Piotr Strzałka
Department of Hematology, Medical University of Lodz,
e-mail: piotr.strzalka@stud.umed.lodz.pl

Introduction

- The biology of the leukemic clone in acute myeloid leukemia (AML) determines resistance to chemotherapy or an increased risk of relapse.
- Enzymatic proteins of the sirtuin family are NAD(+) - dependent histone deacetylases with pleiotropic effects on metabolism, aging and cell survival (Figure 1, Figure 2) [1].

Figure 1. Division of histone deacetylases with particular reference to sirtuins (SIRTs) and their classes.

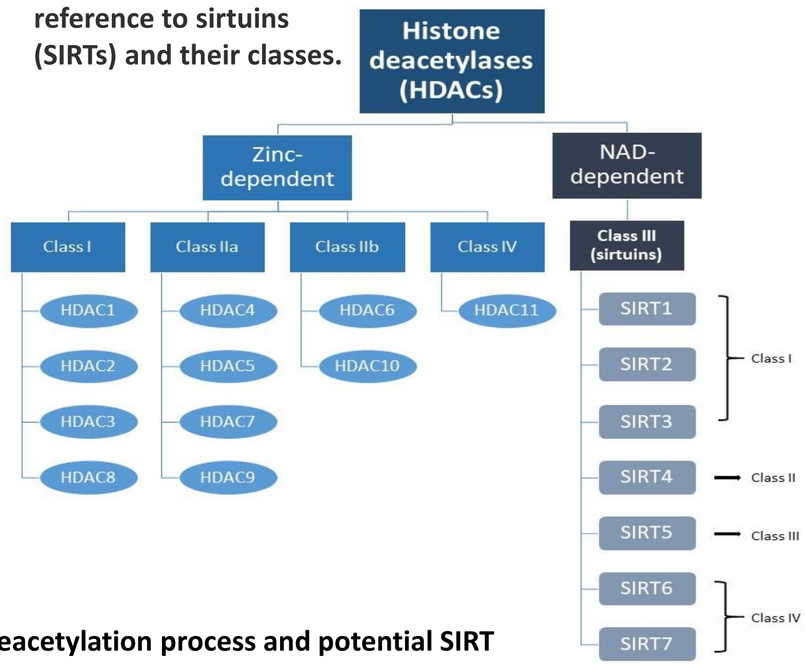
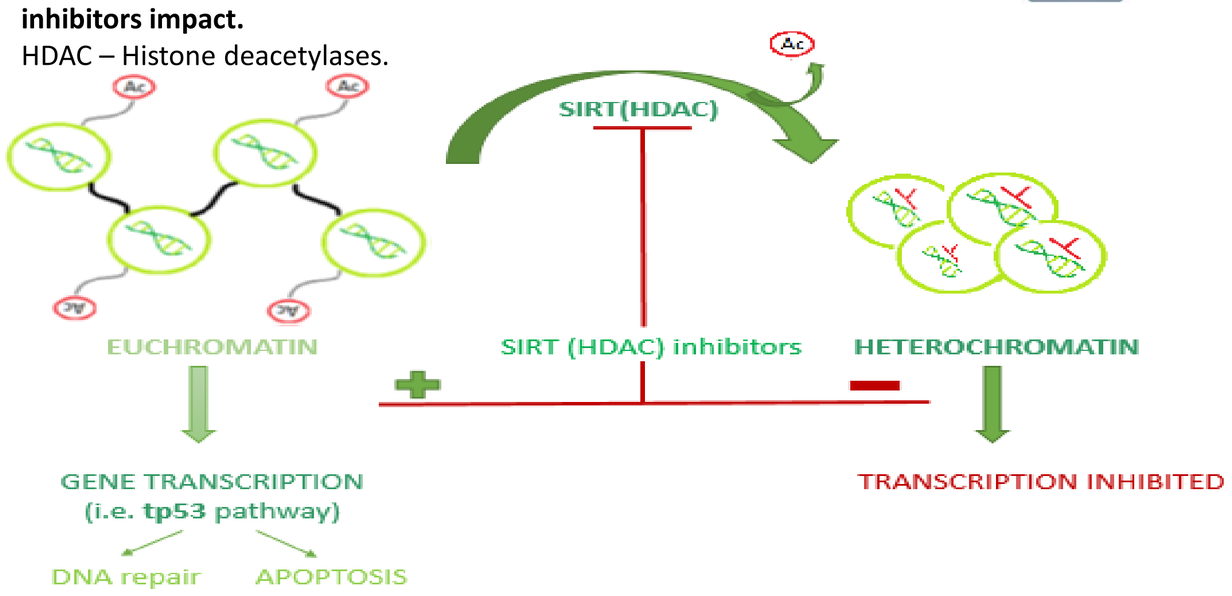
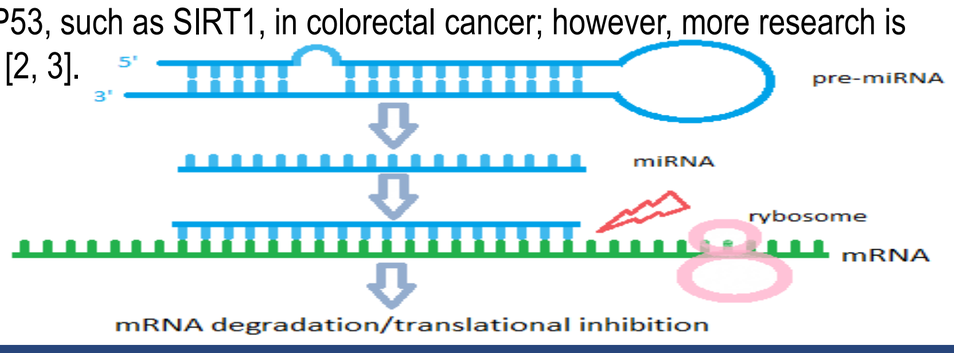


Figure 2. Simplified scheme of deacetylation process and potential SIRT inhibitors impact.
HDAC – Histone deacetylases.



- Another epigenetic mechanism for controlling gene expression, which is related to the action of sirtuins, is microRNA-based regulation (miRNAs) (Figure 3).
- For example, miR-34a can indirectly increase TP53 levels by inhibiting negative regulators of TP53, such as SIRT1, in colorectal cancer; however, more research is needed in AML [2, 3].

Figure 3. A simplified scheme of miRNA processing and their basic functions.

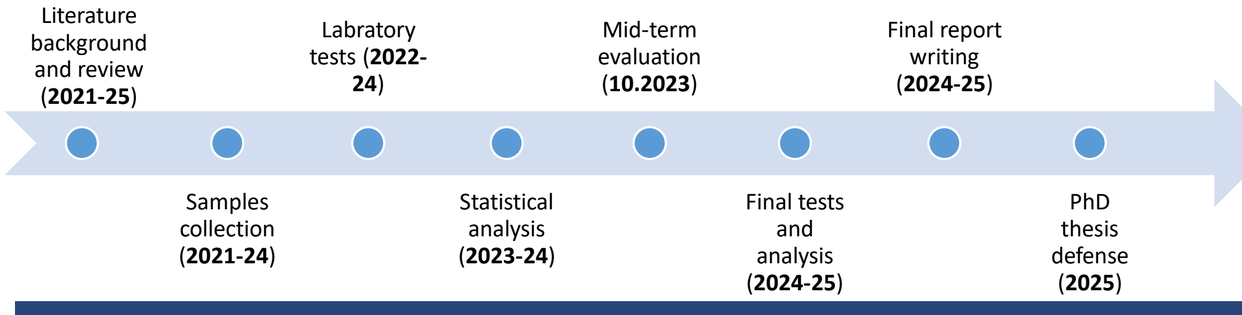


Hypothesis & Aims

Research hypothesis: The expression level of sirtuin family protein genes is important in the biology of acute myeloid leukemia and influences disease course.
Main objective: To comprehensively assess the relationship of individual SIRTs in relation to the clinical course of AML by investigating the expression of *SIRT(1-7)* mRNA, the expression of SIRT-dependent genes (*TP53*), and their association with selected miRNAs.

Materials & Methods

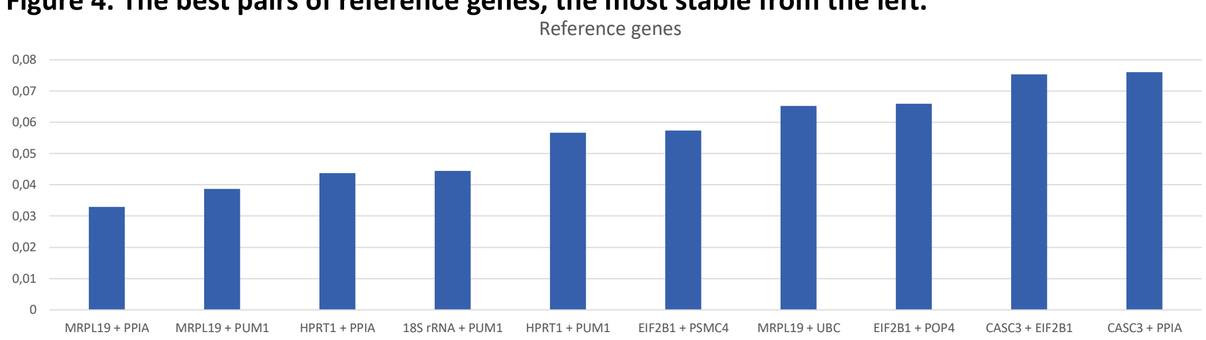
- The prospective, single-centre study is being conducted.
- The study has received a positive opinion from the Bioethics Committee.
- The study assumes the use of genetic material obtained from bone marrow mononuclear cells, collected routinely during hematological diagnosis.
- Patients were included in the study group after giving their informed, voluntary consent to participate in the study, ≥18 years, with newly diagnosed AML.
- Material from 94 patients was banked.
- The mRNA expression of *SIRT1-7* and *TP53* genes, and 14 miRNA molecules (miR-9, miR-10a, miR-26a, miR-34a, miR-125a, miR-125b, miR-132, miR-145, miR-155, miR-181a, miR-204, miR-217, miR-425, miR-486) was determined in duplicates using real-time PCR.



Data Analysis & Results

- Reference genes for the expression of the mRNA were selected based on statistical analysis performed using the Normirazor tool and 3 algorithms: geNorm, BestKeeper, NormFinder. The gene pair *MRPL19* and *PPIA* showed the highest stability (Figure 4).
- For miRNA reference the pair hsa-miR-16-5p and hsa-miR-191-5p was selected as the most stable (preliminary selection based on the literature, the stability of the selected reference genes was statistically confirmed using Normirazor).

Figure 4. The best pairs of reference genes, the most stable from the left.

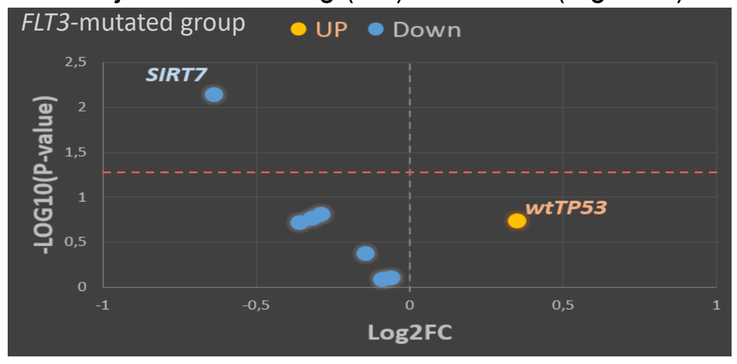


- Normalisation was performed using the formulas ΔCt (treshold cycle) = Ct ([*MRPL19*+*PPIA*]/2) - Ct (mRNAs of interest) and ΔCt = Ct ([hsa-miR-16-5p+hsa-miR-191-5p]/2) - Ct (miRNAs of interest).
- The ΔΔCt method was used to calculate the multiplicity of change in expression.

Results concerning *SIRTs*:

- There were no significant differences in *SIRT(1-7)* expression between the different AML genetic profiles, nor was there effect of their expression on overall survival (OS).
- An association of *wtTP53* expression with prolongation of OS was demonstrated in univariate Cox analysis (HR=0.61, 95% CI: 0.42-0.86, *p*=0.005).
- Sirtuins showed decreased expression in the *FLT3* mutation group, while *wtTP53* showed increased expression. There was a significant difference in the change in mRNA expression multiplicity depending on the presence of *FLT3* mutation for *SIRT7* (FC=0.64, p=0.007). The p-values are given with Benjamini-Hochberg (BH) correction (Figure 5).

Figure 5. Changes in expression multiplicity of the *SIRT* and *wtTP53* genes in the presence of *FLT3* mutations. Up - higher expression, down - lower expression. The red line indicates the cut-off for *p*-value significance.



Results concerning miRNAs:

- Positive correlation was found between miR-9-5p and both *SIRT1* and *SIRT2* (r=0.24, *p*=0.04; r=0.26, *p*=0.02, respectively), and between miR-34a-5p and *wtTP53*, while negative with *SIRT6* (r=0.34, *p*=0.002; r=-0.27, *p*=0.02, respectively).
- Down-regulation was detected of miR-34a-5p in a high-risk group (FC=0.43, *p*(BH)=0.05), and an up-regulation in *FLT3*-mutated group (FC=2.64, *p*(BH)=0.001).
- In a multivariate model adjusted for age, sex, treatment, and risk group miR-125a-5p reduced the probability of treatment response (OR=0.7, 95% CI: 0.48-0.99, *p*=0.05).
- In a multivariate Cox regression, miR-9-5p was negatively affecting OS (HR=1.2, 95% CI: 1.05-1.37, *p*=0.006), while miR-34a-5p had a significant positive impact on survival (HR=0.76, 95% CI: 0.63-0.92, *p*=0.005) in the context of established prognostic factors (Figures 6A-B).
- Clustering of miRNA expression was performed, with separation of two groups by genetic mutation profile and type of AML (Figure 7).

Figure 6. Kaplan-Meier curves for OS depending on miR-9-5p expression (A), and mi-R-34a-5p expression (B); blue line – expression below median, green line – above median.

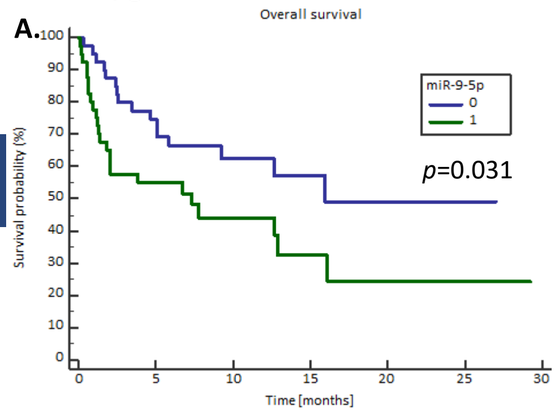
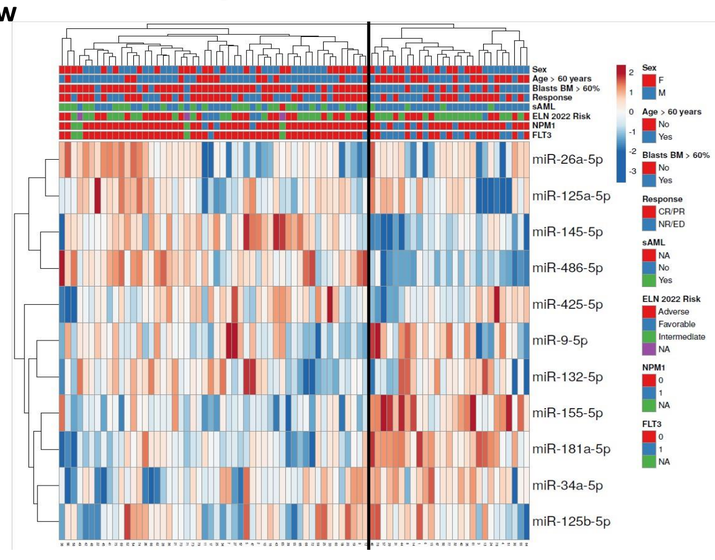


Figure 7. Clustering of miRNA expression according to selected clinical variables.



Conclusions:

The expression of **sirtuins** in AML presents no evident impact on OS. Our preliminary results indicate impaired expression of miRNAs potentially interacting with sirtuins and *TP53* in AML patients. The **miR-9a-5p** and **miR-34a-5p** may act as biomarkers in AML.

References

1. Carafa et. al. Sirtuins function and modulation: from chemistry to the clinic. Clinical Epigenetics. 2016;8:61. doi: 10.1186/s13148-016-0224-3.
2. Gabra MM, Salmena L. microRNAs and Acute Myeloid Leukemia Chemoresistance: A Mechanistic Overview. Front Oncol. 2017;7:255. doi: 10.3389/fonc.2017.00255.
3. Liao Q, Wang B, Li X, et al. miRNAs in acute myeloid leukemia. Oncotarget. 2017;8(2):3666-3682. doi: 10.18632/oncotarget.12343.