Effect of microRNA-100 Expression on differentiated Acute myelocytic Leukemia

Sarah Albahkali [1,2], Ayman Almubarak [2], and Bahauddeen M. Alrfaei [1,3,*]

[1] King Abdullah International Medical Research Center, KSA

[2] King Saud University, College of Science, KSA

[3] King Saud bin Abdulaziz University for Health Sciences ,KSA

Abstract

Background: Acute myeloid leukemia (AML) is the most common malignant disorder of hematopoietic stem cells. The incidence rate of AML is 3.6 in very 100,000 individuals and its mortality 2.7 per 100,000. The leading cause of the disease has not been fully elucidated. miR-100 is known as tumor inhibitor and differentiation molecule solid cancer. However, miR-100 has controversy in liquid tumors.

Objectives: study whether changes in miR-100 expression within AML alter growth or phagocytosis behaviour of AML cells.

Methods and results: miR-100 had been inhibited with lentiviruses in AMoL cell line (THP1) and U937 then viability was calculated. In addition, miR-100 were overexpressed within THP1 and U927 then proliferation was calculated. Moreover, healthy macrophages were co-cultured with miR-100 inhibited AMoL cells which showed that healthy macrophages had killed more leukemia cells when miR-100 was inhibited in it.

Conclusion: miR-100 inhibition within AMoL act as tumor inhibitor but make phagocytosis less efficient. This phenomenon might help us understand weaker against immune system. This means overexpress miR-100 works as supporter to chemotherapy and improve treatment.

Introduction

Leukemia is the reproduction of abnormal white blood cells that disturb the production of functional normal white blood cells resulting in anemia and thrombocytopenia. Leukemia is divided into: Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL) and Chronic Myeloid Leukemia (CML). Acute myeloid leukemia (AML) is the most common malignant disorder of hematopoietic stem cells. The incidence rate of AML is 3.6 in very 100,000 individuals and its mortality 2.7 per 100,000. MicroRNAs (miR) are small, non-coding RNAs (16–22 nucleotides long) that repress protein translation by binding to the 3'UTRs of the target mRNAs gene. The leading cause of the disease has not been fully elucidated. miR-100 is known as tumor inhibitor and differentiation molecule of solid cancer. However, miR-100 has controversy in liquid tumors. Thus, we decided to study the cellular behavior linked to miR-100 in AML subclass acute myelocytic leukemia (AMoL) to clarify whether miR-100 act as tumor suppressor or not.

Methodology

➢ Transfection of miR-100-5p into THP-1 cells and U937.

THP-1 cells were seeded in opaque 96 well plate (10,000 cells/well) in starvation media over night, Next day, transfection reagents were prepared and incubated in room temperature for 25 minutes, then cells were transfected with inhibitor miR-100, mimic miR-100 and negative control for 4 hours at 37C, after that, a media containing 10% serum was added to the cells to allow them to proliferate. Viability was measured by spectrophotometer. In addition, miR-100 was overexpressed within (THP-1) and proliferation was calculated.

➢ Phagocytosis Assay

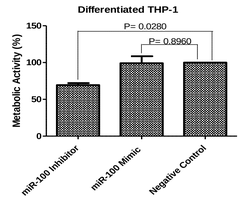
THP-1 cells were seeded in opaque 96 well plate (10,000 cells/well) for 24 hours at 37C.Next day, cells were transfected as described previously. Later, fluorescent bioparticles bacteria were prepared as manufacturer protocol and co-culture with transfected THP-1 cells for 2 hours, after that, bacteria was removed, and viability assay was measured by adding trypan blue for 2 minutes and the results were read by spectrophotometer.

Result

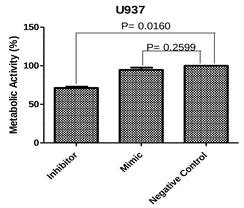
➢Transfection miR-100-5p into THP-1 cells

When miR-100-5p was inhibited and overexpressed in THP-1 cells, there was an inhibition in miR-100 in these cells leading to decrease in the growth, while when was overexpressed, the cells showed increasing in the growth compared to the negative control. **Figure. 1 (A and B)**

(A)



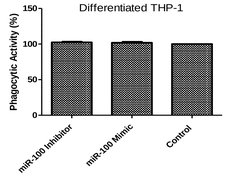
(B)



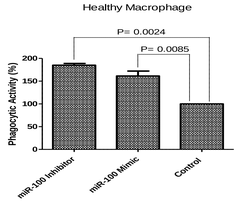
➢Phagocytosis Assay

The phagocytosis activity of differentiated THP-1 showed that there is no effect of miR-100-5p which means there is no phagocytic activity of these cells after differentiation compared to control. **Figure. 2 (A and B)**

(A)



(B)



Conclusion

- THP-1 grows slower when miR-100 is inhibited.
- Over expression of miR-100 did not affect THP-1 growth.
- When miR-100 is inhibited in U937 cells which are lymphoma cells, they showed the same response as THP-1 because they are expressing many of the monocytic like characteristics.
- Phagocytosis is better and higher in healthy macrophages compared to cancer macrophages (derived from THP-1).
- THP-1 has limited ability for phagocytosis even with increased or decreased miR-100 expression. This means miR-100 has no effect on phagocytic process.

References

Zheng, Y. S., et al. "MiR-100 regulates cell differentiation and survival by targeting RBSP3, a phosphatase-like tumor suppressor in acute myeloid leukemia." Oncogene 31.1 (2012): 80-92.

Bai, Jin, et al. "Upregulation of microRNA-100 predicts poor prognosis in patients with pediatric acute myeloid leukemia." OncoTargets and therapy 5 (2012): 213.

Sun, Yin, Hongxiang Wang, and Chibao Luo. "MiR-100 regulates cell viability and apoptosis by targeting ATM in pediatric acute myeloid leukemia." Biochemical and biophysical research communications 522.4 (2020): 855-861.