Expression association of miRNA-103 with acute myeloid leukemia

IV. ULUSLARARASI KATILIMLI DENEYSEL HEMATOLOJİ KONGRE BİLDİRİSİ

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Abstract

Background: Acute myeloid leukemia (AML) is the most common acute leukemia in adults. its incidence increases with ageing. Although it`s etiology unknown, it can develop following exposure to genotoxic agents or following an antecedent hematologic disorder. As a highly heterogeneous disease, AML needs fine risk stratification to get an optimal outcome for patients. The classification systems for AML already incorporate cytogenetic and molecular genetic aberrations in an attempt to better reflect disease biology. miRNA-103, is a small non-coding RNA, located on 5q34, which is up-regulated in different cancers. The aim of this work was to measure the expression level of miRNA-103 in AML.

Methods: The study included 18 cytogenetically normal AML cases and 15 controls, samples were grouped according to the clinical characteristics of patients which include gender and the mean age. Its level was evaluated using quantitative real time polymerase chain reaction (qRT-PCR) technique.

Results: It was revealed that expression level of miRNA-103 significantly up-regulated in AML patients compared to normal controls.

Conclusion: To conclude, increased expression level of the miRNAs might be a risk factor to AML; further studies are mandatory to a better understanding and confirmation our preliminary findings.

Key word : Acute myeloid leukemia, miR-103, qRT-PCR, expression analysis.

INTRODUCTION

Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that build up in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age. Although AML is a relatively rare disease its incidence is expected to increase as the population ages. Several risk factors like smoking, radiation exposure, positive family history, older age, male gender and chromosomal aberrations have been identified, but the specific cause is not clear.[1]

MiRNAs are small, evolutionary conserved, single-stranded, non-coding RNA molecules that bind target mRNAs to prevent protein production by one of two distinct mechanisms. Mature miRNA is generated through two-step cleavage of primary miRNA (pri-miRNA), which incorporates into the effector complex RNA-induced silencing complex (RISC). The miRNA functions as a guide by base-pairing with target mRNA to negatively regulate its expression. The level of complementarity between the guide and mRNA target determines which silencing mechanism will be employed; cleavage of target messenger RNA (mRNA) with subsequent degradation or translation inhibition(Fig. 1). MiRNAs play an important role in cellular differentiation and cancer pathogenesis. Dysregulation of miRNA expression profiles has been demonstrated in most tumors examined .However, the specific classification of miRNA as oncogenes or tumor suppressors can be difficult because of the intricate expression patterns of miRNAs. MiRNAs` expression pattern differ for specific tissues and differentiation states, which poses difficulties in classification . It is not always clear if altered miRNA patterns are the direct cause of the cancer or rather an indirect effect of changes in cellular phenotype. Additionally, a single miRNA can regulate multiple targets.This coupled with tissue specific expression could implicate a single miRNA as a tumor suppressor in one context and an oncogene in another.[2] MiRNA-103, is a small non-coding RNA, located on 5q34, which is up-regulated in different cancers.

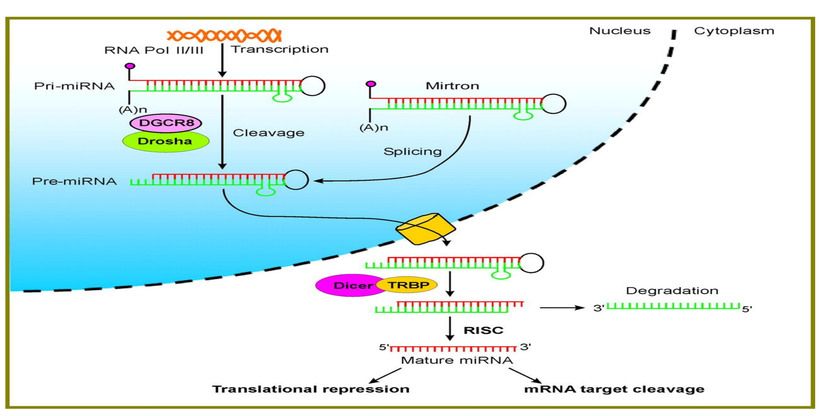

Figure 1. Micro RNA biogenesis and function

MATERIALS AND METHODS

Patients

The study included 18 cytogenetically normal AML cases and 15 controls, samples were grouped according to the clinical characteristics of patients which include gender and the mean age.

Micro-RNA Extraction

MiRNAs were extracted from peripheral blood samples using miRNA extraction kit (InvetrogenTM / life technologies/ USA). The miRNA at the end was eluted by 50-100 µl of nuclease free water. The miRNA concentration was measured using NanoDrop-1000 spectro-photometer (Nano Drop Technologies, USA).

Converting cDNA and real-time PCR using TaqMan

The extracted miRNA was reversed transcribed using TaqMan microRNA reverse transcription kit (ApplidBiosystems, USA) according to the manufacture protocol. Then 5 µl of miRNA was reversed transcribed in a 15 µl reaction volume for each assay. TaqMan miRNA-103 probe was used to quantify miRNA in real time PCR assays according to manufacture protocol. Real time PCR assays were performed in a 20 µl reaction volume.

Statistical analysis

The relative quantitative gene expression level was evaluated using the ΔΔCt comparative Ct method. Fold inductions were calculated using the formula 2^ (ΔΔCt) where:

ΔCt=Ct (target gene)-Ct (reference gene)

ΔΔCt=ΔCt (treated) − ΔCt (control).

RESULTS

The expression level of miRNA-103 gene was obtained from 18 cytogenetically normal AML cases and 15 controls; quantity of expression of miRNA-103 in AML samples was increased (up-regulated) according to expression level of normal control samples, p=0,002 and statistically it is significant counted on (p < 0,05). The miRNA-103 expression levels for both normal controls and AML cases are shown in Figure 2.

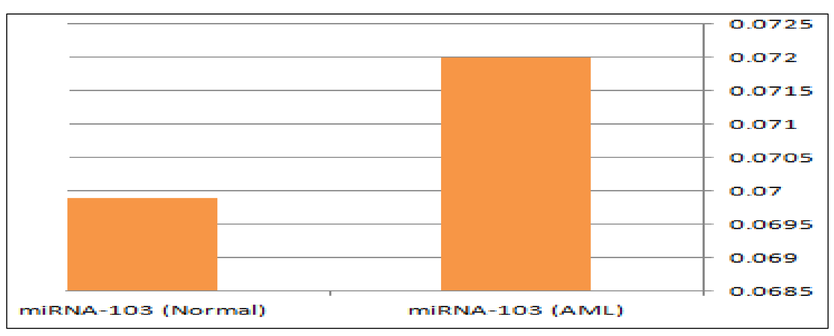

Figure 2. The miRNA-103 expression levels for normal controls and AML

CONCLUSION

To conclude, increased expression level of the miRNA might be a risk factor for AML; further studies are mandatory for a better understanding and confirmation of our preliminary findings.

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