Alterations of certain microRNA expressions in febrile neutropenia patients

Tolnai E¹, Fidler G¹, Nwozor K¹, Tóth A¹, Szász R², Rejtő L², Biró S¹, Paholcsek M¹

¹University of Debrecen, Faculty of Medicine, Department of Human Genetics, Debrecen, Hungary

²University of Debrecen, Institute of Internal Medicine, Division of Haematology, Debrecen, Hungary

Introduction

MicroRNAs (miRNAs) are small 19- to 23- nucleotide long, single-stranded RNA molecules. MiRNAs have been shown to be involved in various biological processes, like cell proliferation, differentiation, apoptosis, inflammation and cancer. Aberrant expression of miRNAs has been associated in many diseases. Accumulating evidence demonstrates that specific miRNAs can be a great promise as novel biomarkers for clinical diagnosis of many types of diseases.

Invasive aspergillosis (IA) is a life-threatening infection caused by *Aspergillus* especially in immunocompromised patients. Regrettably, the gold-standard diagnostic setups for this condition take days to produce a reliable results.

Objectives

Our aim was to measure the expression profiles of 19 selected miRNAs in 16 patients with febrile neutropenia (FN) and in further 5 non neutropenic healthy volunteers. In this study we hypothesized that IA could change the miRNA profiles in whole blood (WB) of patients with FN provoked by IA.

Methods

We obtained blood samples from 16 patients suffering neutropenic fever and from 5 healthy volunteers without fever. We extracted total RNA from whole blood using the Magmax mirVana Total RNA isolation Kit (Thermo Fisher Scientific). MiRNAs were reverse transcribed to cDNA with TaqMan advanced miRNA cDNA Synthesis kit (Thermo Fisher Scientific). TaqMan quantitative real-time PCR (miRNA assay, Thermo Fisher Scientific) was used for detecting miRNA expression profiles. Relative miRNA expressions were calculated with the $\Delta\Delta C_t$ method using *mir191* as internal control. Febrile neutropenia patients were classified according to revised European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria in order to define a case group of „proven” or „probable” IA (8 patients) and control group of „possible” IA (8 patients with no evidence of IA) and 5 healthy volunteers.

Results

A total of 19 miRNAs were analysed in the case and control groups

Figure 1: Showing the expression levels of the investigated genes. P-values were calculated using two-tailed Student's *t*-test for samples of unequal variance; ($p < 0.05$) * was used to indicate level of significance.

7 miRNAs showed statistically significant upregulation compared to the control group

Figure 2: miRNAs with significant upregulations in the case group compared to the control group were shown in a higher scale for better visibility. (a) miR-140-5p (b) miR-26a-5p (c) miR-26b-5p (d) miR-142-3p (e) miR-21-5p (f) miR-101-3p (g) miR-16-5p.

4 miRNAs were able to lay down distinct diagnostic windows

Figure 3: Whisker plots showing the distribution of the absolute copy number of the miRNAs which were derived from the normalized reciprocal *Ct* values measured for target miRNA versus miR-191. In the case of miR-26-5p, miR-21-5p, miR-26b-5p and miR-142-3p, we were able to allocate distinct diagnostic windows.

Conclusions

Based on the result of this study, seven miRNAs with significant different expression between the test group and the control group may be an effective biomarker targets when monitoring patients with FN to support the diagnosis of IA. Furthermore, in the case of 4 miRNA we were able to distinguish diagnostic windows using normalized *Ct* values to discriminate between cases and controls. Our goal is to make a rapid test supporting diagnosis of IA.