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Short Communication

Sequence comparison of six human microRNAs genes between tuberculosis patients and healthy individuals

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ABSTRACT

Objective/Background: MicroRNAs (miRNAs) play an important role in diseases development. Therefore, human miRNAs may be able to inhibit the survival of *Mycobacterium tuberculosis* (Mtb) in the human host by targeting critical genes of the pathogen. Mutations within miRNAs can alter their target selection, thereby preventing them from inhibiting Mtb genes, thus increasing host susceptibility to the disease.

Methods: This study was undertaken to investigate the genetic association of pulmonary tuberculosis (TB) with six human miRNAs genes, namely, *hsa-miR-370*, *hsa-miR-520d*, *hsa-miR-154*, *hsa-miR-497*, *hsa-miR-758*, and *hsa-miR-593*, which have been predicted to interact with Mtb genes. The objective of the study was to determine the possible sequence variation of selected miRNA genes that are potentially associated with the inhibition of critical Mtb genes in TB patients.

Results: The study did not show differences in the sequences compared with healthy individuals without antecedents of TB.

Conclusion: This result could have been influenced by the sample size and the selection of miRNA genes, which need to be addressed in future studies.

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) is commonly manifested as a pulmonary disease [1,2]. Estimates indicate that 8.8 million new cases of active TB and 1.4 million deaths are caused by this disease every year. One third of the human population is latently infected with Mtb [1,2]. Only approximately 10% of infected individuals develop the disease, and the remaining 90% do not progress to clinical TB, although the bacteria remains

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in the dormant state and can be reactivated at any time [1,2].

Pathogenic and environmental factors influence host's susceptibility to TB and host genetic factors also contribute to resistance/susceptibility to this disease [3–6].

One of the elements of great importance in gene expression and regulation is the microRNAs (miRNAs) [7]. Mature miRNAs (20–22 nt) are formed from long hairpins containing miRNA precursors (pre-miRNAs; 70–100 nt) as small single-stranded noncoding RNAs controlling the expression levels of their target genes by messenger RNA (mRNA) degradation and protein translation inhibition [7].

There are currently about 1000 human miRNA sequences available in the miRNA registry representing approximately 60% of all mammalian genes [8]. This indicates that miRNAs are involved in the fundamental and global functions in human biology. The miRNAs are also known to be potential regulators of immune reactivity [9].

Interindividual differences of miRNAs expression are likely to influence the expression of miRNA target genes, contributing to some changes in human phenotype, conditioning the evolution, and influencing the susceptibility/resistance to multiple diseases [9]. The association of miRNAs with multiple diseases, including infectious diseases, has been extensively studied [9–12].

The potential role of miRNAs in the susceptibility and evolution of TB has been studied extensively, with reports indicating changes in several miRNAs, highlighting the potential role of these molecules in the disease [13–15].

In general, the main alterations related with TB have been found associated with miRNAs involved in the regulation of the immune responses and inflammation [13–15].

The potential impact resulting from interactions of human miRNAs with the expression of Mtb genes has not yet been explored. Human miRNAs directed at critical genes of Mtb associated with virulence and survival of the bacteria could have an important role in the control of the infection. Mutations in such host miRNA genes could abolish the inhibition of the expression of critical Mtb genes and increase the host's susceptibility to the disease. Guo et al. [16] predicted 26 candidate Mtb genes that are expressed in macrophages and in the lungs of humans and mice that may be targeted by 31 human miRNAs [16]. These 31 human miRNAs were selected as they are expressed in the lungs and macrophages based on miRNAs expression atlas [17].

In this study, we selected six of the miRNAs predicted by Guo et al. [16], which target Mtb genes that are responsible for the virulence and survival of Mtb. These genes have been reported to be expressed *in vivo* and/or in macrophages in culture [18–22]. The objective of this study was to explore possible polymorphisms within these miRNAs using DNA sequencing.

Materials and methods

Study population

Newly diagnosed adult pulmonary TB patients ($n = 33$) from the Hospital Universiti Sains Malaysia (Kubang Kerian, Kelantan, Malaysia) and healthy individuals without TB antecedents ($n = 38$) were included in the study. A peripheral blood sample from each participant was collected into a Vacutainer tube containing the anticoagulant EDTA after obtaining informed consent from each participant. This study was approved by the Human Ethics Committee of Universiti Sains Malaysia.

Selection of miRNAs

Thirty-one human miRNAs expressed in the lungs and macrophages and their 26 potential target Mtb genes were previously predicted by Guo et al. [16]. From this previous study, we chose to study six human miRNAs, which are predicted to target five important Mtb genes, using their overexpression after infection in mice (lung and artificial granuloma) [18,19] and/or humans (lung, sputum, and macrophage cell lines) as the main selection criterion [20,22], because they may be important in virulence/survival of Mtb *in vivo*.

Primer design

Primers were designed to flank the sites of miRNAs using NCBI Primer-Basic Local Alignment Search Tool. Hairpin formation, primer dimerization, and self-primer dimerization were carefully inspected using an online software from integrated DNA Technologies. Each primer set was designed to amplify the amplicon containing the sequence of specific miRNA to give a bigger product than the pre-miRNA to ensure that the full pre-miRNA sequence is obtained. The gene sequence of pre-miRNAs of each miRNA was obtained from the NCBI reference sequence [23].

Polymerase chain reaction

Genomic DNA was extracted using the QIAamp DNA blood mini kit (QIAGEN, Germantown, Maryland, USA). Polymerase chain reaction (PCR) was performed using specific primers for each miRNA. The PCR was performed in a 20-μL reaction mix comprising 10 pmol of each primer (Sigma–Aldrich, Singapore), 2 μL of 10 × *Taq* reaction buffer, and 3 μL of deoxynucleotide triphosphate mix (2.5 mM each; Geneall Biotechnology, Seoul, Korea).

The mixture was initially heated at 95 °C for 3 min to activate the polymerase. The DNA amplification was performed for 30 cycles as follows: at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 50 s. The final elongation step was performed at 72 °C for 10 min and the reaction was held at 4 °C. PCR products were purified and sequenced using the ABI 3130xl genetic analyzer.

DNA sequencing

Termination cycle

The termination cycle of the purified PCR product was carried out in a final volume of 10 μL in a 0.5-mL tube containing 3 μL of the purified PCR product, 3.75 μL of distilled water, 1 μL of primer (each tube contained only 1 type of primer F1 or R1), 1.75 μL of 5× buffer, and 0.5 μL of big dye (Applied Biosystems, Carlsbad, California, USA). The cycle sequence amplification