microRNA as Potential Biomarker for Pediatric Tuberculosis?

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Abstract

The diagnosis of pediatric TB is based on history taking, clinical symptoms, physical examination and support. In recent years the role of microRNA (miRNA or miR) has become a concern for researchers as biomarkers of diagnosis and therapy in TB in adults and children. MicroRNA is a ribonucleic acid that does not encode proteins with 18-25 nucleotide transcripts that interact with gene targets and regulate mRNA expression. miRNA works with other regulatory elements such as transcription factors to control mRNA translation. More than 100 different miRNAs are expressed by immune system cells; they have the potential to broadly influence the molecular pathways that control the development and function of innate and adaptive immune response regulation. During TB infection, the innate immune response provides an initial defense mechanism against infection. It is well known that macrophages are the main stem cells for mycobacteria, survival in macrophages is determined by host-pathogen interactions. Several studies have shown that miRNA can be used as a biomarker and TB therapy agent because it is stable in plasma and other body fluids, difficult to degrade and excreted in the form of exosomes or micro vesicles. Other studies say miRNA is stable despite repeated exposure to heat, cold, acids, bases, and other extreme conditions. microRNA levels are reported to be increased in individuals with TB.

***Keywords**: pediatric tuberculosis; diagnosis; therapy; microRNA; biomarker*

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by infection with Mycobacterium tuberculosis (M.tb). This disease is the second leading cause of death in the world due to infection after HIV / AIDS[1,2]. It is estimated that around two billion people suffer from latent TB infection and cause new TB cases in 9.2 million people and deaths in 1.7 million people in the world. About 5-10% of people who suffer from latent TB infection will become active TB in the first five years after becoming infected with TB germs[3,4]. In 2015 there were 10.4 million new TB cases worldwide, 10% of which were pediatric TB[5].

The diagnosis of pediatric TB is based on history taking, clinical symptoms, physical examination and support. However, children infected with TB often show typical symptoms[6,7]. Tuberculin testing based on cellular immunity is considered impractical because it requires a minimum of two diagnostic visits. Tuberculin test can be affected by various conditions so that it can produce false positives or false negatives[6–8]. Other investigations that are believed to be used in the diagnosis of TB include Interferon Gamma Release Assay (IGRA). The use of IGRA for the diagnosis of TB in children is still limited due to the high cost of examinations and the lack of examination tools and techniques. This examination still cannot distinguish active and latent TB infections[9,10].

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The definitive diagnosis of TB is made by finding TB germs on direct smear examination and culture which is a gold standard examination. But a definitive diagnosis in children is difficult to obtain because of the small number of germs (pausibasiler), the child is difficult to expel phlegm, the location of germs in the parenchyma area far from the bronchus. Indonesia has developed a TB scoring system for children and is still being applied to help establish a diagnosis of TB in children[7,8,10,11].

In recent years the role of miRNA has become a concern for researchers as biomarkers of diagnosis and therapy in TB in adults and children. Identification/ profiling studies of miRNA in TB with microarray profiling have been carried out in countries. There are significant differences between levels of miRNA expression in pediatric TB patients compared with healthy children and have significant diagnostic values. This suggests that miRNA can be one of the tools to diagnose TB in children that needs to be developed[9,12-20].

microRNA

microRNA is a ribonucleic acid that does not encode proteins with 18-25 nucleotide final transcripts that interact with gene targets and regulate mRNA expression. miRNA works with other regulatory elements such as transcription factors to control mRNA translation. Most miRNAs are encoded in parts of the introns that used to be considered non-coding regions. The miRNA genes are distributed in the genome and are estimated to make up 2-5% of the human genes. miRNA is often expressed as a polysistronic transcript. One miRNA can have many target mRNAs so it is estimated that more than 1/3 of human genes are regulated by miRNA[13,20,21].

More than 100 different miRNAs are expressed by immune system cells; they have the potential to broadly influence the molecular pathways that control the development and function of innate and adaptive immune response regulation. Apart from impaired immune function, miRNA is also known to play a role in infection. microRNA plays an important role in cell processes such as cell differentiation, cell cycle, embryonic development, apoptosis and associated with infectious diseases including TB[22,23]. Research has shown that stable miRNA serum faces repeated freezing and thawing as well as heat, acid and base conditions and other extreme conditions. This has the potential to be a useful biomarker for disease diagnosis, the effects of therapy and prognosis[20].

microRNA Biogenesis

microRNA is a small, encoded RNA molecule that regulates several biological processes by interfering with mRNA translation. miRNA biosynthesis through a process involving several proteins and enzymes found in the nucleus and cytoplasm. miRNA biosynthesis originates from the miRNA gene in the nucleus where the main miRNA undergoes transcription as a large double-chain primary transcript called pri-miRNA by RNA polymerase II. Pri-miRNA folded into structures such as hair pins undergo polyadenylation and capped. The Drosha RNAse type III enzyme converts this precursor into a double chain miRNA precursor from 60 to 100-nt hairpin known as pre-miRNA. Pre-miRNA consists of a local stem-loop structure that encodes the miRNA sequence that is exported from the nucleus to the cytoplasm by exportin 5[9,23].

In the cytoplasm, pre-miRNA is further processed by Dicer RNAse III to become duplex miRNA. This unstable duplex consists of guide strand (miRNA) and passenger strand (miRNA *). The miRNA strand guide chain will become mature miRNA, where miRNA * is degraded. Mature miRNA is facilitated by Argonaut protein incorporated into RNA-induced silencing complex (RISC), which recognizes specific miRNA targets and triggers post-transcriptional silencing genes to regulate protein expression, target cleavage mRNAs, suppress / inhibit translation processes, and deadenylate mRNA[9,22].

The Role of microRNA in Tuberculosis

Studies show that different miRNAs play a major role in the process of cell differentiation involved in protection against bacterial infections, such as differentiation and function of immune cells, control of chemokine production and regulation of the immune response. miRNA regulates gene expression by targeting mRNA3'-regions that are not translated, modifying translation and / or degradation. During TB infection, the innate immune response provides an initial defense mechanism against infection. It is well known that macrophages are the main stem cells for mycobacteria, survival in macrophages is determined by host-pathogen interactions[24-31].

Study found disturbed gene expression profiles in macrophages and NK cells from active TB, latent TB and healthy controls that appeared to be regulated by miRNA. Some miRNAs play a role in the regulation of

T cell differentiation and function. Research shows that miRNA plays an important role in regulating the innate functions of macrophages, DC and NK cells (12,32). Hundreds of miRNA encoded in the human genome and thousands of mRNAs have been shown to be involved in cell development, differentiation, proliferation, apotosis, DNA methylation, DNA repair and regulating anti-inflammatory or pro-inflammatory stimuli[33].

Research by Zhou found 14 miRNAs that are very important in pediatric TB. RT-qPCR validates that miRNA-1, miRNA-155, miRNA-31, miRNA-146a, miRNA-10a, miRNA-125b and miRNA-150 are downregulated while miRNA-29 experiences upregulation in children with TB compared to children with TB not infected[20].

Modulating the expression of miRNA-125b, which, in turn, will reduce the level of TNF-α and other major cytokines for controlling M.tb. The targets of miRNA-132 and miRNA-26a show a mechanism by which M.tb can limit macrophage responses to IFN-y by interfering with the expression of host miRNA[35]. A study reported that miRNA-21 inhibits the expression of proinflammatory cytokines and increases the production of anti-inflammatory cytokines, IL-10. miRNA-21 is upregulated in un-sensitized DC and macrophages via the TLR/Erk/NF-κB pathway and also regulated following macrophages together with M.Tb which secretes 6 kDa protein (ESAT-6). Inhibition of IL-12 expression by targeting 3'UTR IL-12 mRNA directly and suppressing Th1 response[2,36].

Clinical and in vitro studies note that miRNA-29 is over-expressed after M.Tb infection in several types of human cells. miRNA-29 suppresses the immune response to M.Tb by downregulating IFN-γ. Besides targeting IFN-′ mRNA 3′UTR, miRNA-29 forms an IFN-R mRNA relationship with Argonaut 2 protein (Ago2) to form RNA-induced silencing complexes and subsequently suppress IFN-γ expression post-transcriptionally. Several studies indicate that miRNA-29 also targets anti-apoptosis B-cell lymphoma 2 (Bcl-2) proteins and regulates the apoptotic pathway in immune cells[16,18,37,38]. Understanding the expression patterns and regulation of miRNA in active TB and latent TB infection opens the possibility that miRNA can be used as a potential diagnostic marker candidate for TB.

microRNA as a Biomarker in Tuberculosis

Early diagnosis is important in efforts to control or effectively treat TB. The heterogeneous clinical presentation of M.tb infection (active TB, latent asymptomatic TB, pulmonary TB, and extra pulmonary TB) is a reason for the development of diagnostic biomarkers. It aims to improve the quality of diagnostic papal bacillary TB or TB cases that are difficult to ascertain for example in children, individuals with HIV, extra pulmonary cases.

The mechanism of LTBI and its transition to active TB is still unclear. Many studies provide evidence that this transition arises if cell-mediated immunity fails. Previous studies have shown that gene expression profile is disrupted in macrophages and NK cells from active tuberculosis and LTBI is thought to have a major role in controlling miRNA expression and describing significant markers for knowing and diagnosing active tuberculosis and LTBI[39].

Sputum, serum, plasma, or other body fluid specimens can be used for non-invasive miRNA analysis, in addition the level of miRNA expression seems stable and can be reproduced in serum, which makes them a potential marker for disease diagnosis so that miRNA can be considered a biomarker which is ideal for TB disease diagnosis[40].

Overall previous research revealed the role of miRNA in the immune and inflammatory response to TB. There is evidence that circulating miRNA exerts biological functions as part of intercellular communication and can be used as biomarkers for human disease. The attractiveness of using plasma miRNA in clinical applications is very high, because the separation and storage of plasma or serum samples is already a clinical routine in TB endemic countries[19].

Several studies have shown that miRNA can be used as a biomarker and TB therapy agent because it is stable in plasma and other body fluids, difficult to degrade and excreted in the form of exosomes or micro vesicles. Other studies say miRNA is stable despite repeated exposure to heat, cold, acids, bases, and other extreme conditions. MiRNA levels are reported to be increased in individuals with TB[14,17]. Studies conducted on children show that there are significant differences between miRNA levels in children with TB compared to healthy children and have significant diagnostic values. This shows that miRNA can be one of the tools to diagnose TB in children that needs to be developed[15,18].

Additional Informations

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