Potential Diagnostic Power of Blood Circular RNA Expression in Active Pulmonary Tuberculosis

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ABSTRACT

Background: Circular RNAs (circRNAs) are a class of novel RNAs with important biological functions, and aberrant expression of circRNAs has been implicated in human diseases. However, the feasibility of using blood circRNAs as disease biomarkers is largely unknown.

Methods: We explored the potential of using human peripheral blood mononuclear cell (PBMC) circRNAs as marker molecules to diagnose active pulmonary tuberculosis (TB).

Findings: First, we demonstrated that circRNAs are widely expressed in human PBMCs and that many are abundant enough to be detected. Second, we found that the magnitude of PBMC circRNAs in TB patients was higher than that in the paired healthy controls. Compared with host linear transcripts, the circRNAs within several pathways are disproportionately upregulated in active TB patients, including “Cytokine-cytokine receptor interaction”, “Chemokine signaling pathway”, “Neurotrophin signaling pathway”, and “Bacterial invasion of epithelial cells”. Based on the differentially expressed circRNAs within these pathways, we developed a PBMC circRNA-based molecular signature differentiating active TB patients from healthy controls. We validated the classification power of the PBMC circRNA signature in an independent cohort with the area under the receiver operating characteristic curve (AUC) at 0.946.

Interpretation: Our results suggest that PBMC circRNAs are potentially reliable marker molecules in TB diagnosis.

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1. Introduction

Circular RNAs (circRNAs) are a class of novel RNAs that are expressed across the eukaryotic tree of life (Jeck and Sharpless, 2014; Wang et al., 2014). CircRNAs can be formed by back-splicing, which is a non-canonical process in RNA splicing (Jeck and Sharpless, 2014). Although little is known regarding the exact mechanisms of circRNA biogenesis, some factors have been shown to regulate this process, such as flank intronic sequences (Liang and Wilusz, 2014; Vicens and Westhof, 2014; Zhang et al., 2014) and RNA-binding proteins (Barrett et al., 2015; Qu et al., 2015). CircRNAs have been revealed to perform several important functions, including microRNA (miRNA) sponges (Hansen et al., 2013; Memczak et al., 2013), alternative RNA splicing regulation (Conn et al., 2017), and transcriptional regulation of the parental gene (Li et al., 2015b). Recent studies have also suggested that some circRNAs can be translated in a cap-independent translation manner and function in myogenesis (Legnini et al., 2017; Pamudurti et al., 2017; Yang et al., 2017). Furthermore, circRNAs have been found to be differentially expressed across human tissues and cell types (Guo et al., 2014; Salzman et al., 2013) and extraordinarily enriched in the mammalian brain (Rybak-Wolf et al., 2015; You et al., 2015). Although circRNAs are generally expressed at low levels (Chen, 2016; Guo et al., 2014), some circRNAs are expressed more abundantly than their linear counterparts (Salzman et al., 2013). Dynamic circRNA profiles are related to neurogenesis (Rybak-Wolf et al., 2015), mouse brain development

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(You et al., 2015), and human epithelial-mesenchymal transition (Conn et al., 2015). Aberrant expression of many circRNAs has been implicated in several human diseases, including cancers (Meng et al., 2017), neurodegenerative diseases (Kumar et al., 2017), and some hematological malignancies (Bonizzotto et al., 2016). Notably, circRNAs are enriched and stable in whole blood (Memczak et al., 2015), platelets (Alhasan et al., 2016), and exosomes (Li et al., 2015a). These features make circRNAs in human peripheral blood good candidates for diagnostic or prognostic biomarkers of human diseases.

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (Pai et al., 2016). Although TB incidence, prevalence, and mortality have decreased since 2000, the World Health Organization (WHO) estimated that there were 1.5 million TB-associated deaths and 10.5 million new TB cases worldwide in 2015 (World Health Organization, 2016). In particular, China has the third largest number of TB cases in the world, which is approximately 10% of the world total (World Health Organization, 2016). To achieve better TB management, several novel diagnostic methods, especially a rapid, low-cost, and non-sputum-based test, are required to screen active TB patients at the primary-care level (World Health Organization, 2016). Recently, several studies have developed blood transcriptomic signatures to distinguish active pulmonary TB patients from other pulmonary disease cohorts and healthy controls (Bloom et al., 2013; Qian et al., 2016; Roe et al., 2016; Sweeney et al., 2016) and predicted the risk of developing active TB (Zak et al., 2016). However, the expression of mRNA transcripts in peripheral blood can be obscured by blood collection procedures, which may slow down the process of their clinical applications (Dvinge et al., 2014). As an alternative to blood transcriptional signatures, Liu et al. also developed a method of quantifying the circulating Mycobacterium tuberculosis antigen peptides for active TB diagnosis and treatment monitoring (Liu et al., 2017). Compared with blood transcriptional signatures, the amount of circulating bacterial antigens in blood could be too low to be detected at the early stage of disease (Abbosh et al., 2017). Therefore, new markers in the peripheral blood are needed to facilitate the early diagnosis and treatment of active pulmonary TB.

In this study, we investigated the potential of using circRNAs in peripheral blood mononuclear cells (PBMCs) as biomarkers for pulmonary TB diagnosis. First, we characterized circRNA expression profiles in human PBMCs using ribosomal RNA-depleted (rRNA-depleted) RNA-seq and circRNA expression microarrays. We compared the circRNA repertoire in PBMCs with that in whole blood and other blood components, such as platelets and red blood cells. Under the assumption that the function of a given circRNA could be associated with the known function of its parental gene, we performed a pathway-based analysis to identify the PBMC circRNAs that are dysregulated in TB patients. Compared with host linear transcripts, the circRNAs within several Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) physiological pathways were observed to be disproportionately upregulated in active TB patients. Based on these differentially expressed circRNAs, we developed a PBMC circRNA-based molecular signature that can differentiate active TB patients from healthy controls. Finally, we validated the performance of our circRNA signature in an independent cohort using quantitative real-time PCR (qRT-PCR). Our results suggest the feasibility of using PBMC circRNAs as molecular markers to diagnose active TB. Fig. 1 provides an overview of the experimental design.

2. Materials and Methods

2.1. Human Subjects

This study was approved by the Ethics Committee of Bengbu Medical College, with written informed consent obtained from all subjects. This was conformed to standards indicated by the Declaration of Helsinki. The diagnosis of active pulmonary TB was based on established international guidelines (Lewinsohn et al., 2017). Subjects with other concurrent infectious diseases were excluded. All the subjects investigated were of Chinese Han descent. The discovery cohort included two subjects with active pulmonary TB and two age- and gender-matched healthy controls: one young male case-control pair and one senior female case-control pair (Supplementary Table S1). The validation cohort consisted of 10 TB patients and 11 healthy controls, with no significant difference in age and gender between the cases and controls (Supplementary Table S2). The TB patients in the discovery and validation cohorts were recruited from the First Hospital of Huainan City and the Infectious Disease Hospital of Bengbu City, respectively. The healthy controls were recruited from Bengbu Medical College. Several clinical TB status indicators of each individual in the validation cohort, including the number of cavities, diameter of the largest cavities, and sputum smear grade were obtained.

2.2. Cell Purification, RNA Isolation and circRNA Expression Profiling

PBMCs were collected from all subjects in the discovery and validation cohorts. To profile PBMC circRNA expression in the discovery cohort, we constructed ribosomal RNA (rRNA)-depleted RNA-seq libraries using PBMC total RNA from each person in the discovery cohort. The expression of PBMC circRNAs in the discovery cohort was also profiled using the CapitalBio Technology Human CircRNA Array v2 (CapitalBio Technology, Beijing, China). The experimental details of cell purification, RNA isolation, RNA-seq library preparation and sequencing, and circRNA microarray expression profiling were available in Supplementary Methods. Raw sequencing reads were available at the NCBI Short Read Archive (SRA) (Kodama et al., 2012) under the access number SRP115429, and circRNA microarray data were deposited at the NCBI Gene Expression Omnibus (GEO) (Edgar et al., 2002) under the accession number GSE103188.

2.3. RNA-seq Data of Whole Blood and Other Blood Components

To compare the circRNA repertoire in PBMCs with that in whole blood and other blood components, we collected several RNA-seq datasets from public databases, which included six samples of human whole blood (Memczak et al., 2015), three samples of human blood platelets (Kissopoulou et al., 2013), and one sample of human red blood cells (RBCs) (Alhasan et al., 2016) (Supplementary Table S3). All these samples were sequenced using rRNA-depleted RNA-seq libraries in their original studies (Alhasan et al., 2016; Kissopoulou et al., 2013; Memczak et al., 2015). We downloaded the raw RNA-seq reads of these samples from SRA (Kodama et al., 2012).

2.4. Identification and Expression Quantification of circRNAs From RNA-seq Data

The raw RNA-seq reads were filtered by removing adaptor sequences, contamination, and low-quality reads. To assure the sequencing performance and library quality, we used RNA-SeQC (DeLuca et al., 2012) to assess the data quality of each RNA-seq dataset. For each sample, we identified all putative circRNAs using CIRI (Gao et al., 2015) with default parameter settings. After circRNA identification, we quantified the expression values of all identified circular transcripts and known linear transcripts in the Ensembl (Cunningham et al., 2015) human gene annotation (release 87) using our recent developed tool, Sailsfish-cir (Li et al., 2017), with default settings. For each host gene, the transcripts per million (TPM) values of both linear and circular transcripts were recorded. These circRNA identification and quantification procedures were performed for both our PBMC and the public (the other blood component) RNA-seq raw data. The normalized TPM values of circRNAs were used to explore the global differences of circRNA repertoire among human blood components.
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