



Genetic diversity of antigen 38 kDa in *Mycobacterium tuberculosis* strains from China

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ABSTRACT

We used 335 *Mycobacterium tuberculosis* strains from 2010 National Epidemiologic Survey for TB in China and performed comparative sequence analysis of 38 kDa gene after amplification. From the results, we found that there were 5.07% *M. tuberculosis* strains that demonstrated genetic diversity of 38 kDa in China, and 2.99% strains showed polymorphism of the 38 kDa antigen, and this may be the reason for changes in the antigen produced, which may in turn cause alterations of related functions, thereby allowing immune evasion.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a major public health issue, particularly in developing countries. China has the second highest TB burden in the world, with an estimated 1.4 million new TB cases annually and more than 44.5% of the population infected with *M. tuberculosis* [1]. Current efforts to reduce this global problem focus on improving diagnosis and developing effective vaccines [2]. 38 kDa (Rv0934, PstS1) is one of the most immunogenic antigens and a good candidate for the diagnosis and vaccination against tuberculosis [3–6]. Previous studies showed that the serologically immunodominant epitope of the 38 kDa is conformational rather than linear [7] and some mutations occurred in the antigen 38 kDa, which may lead to alternation in the protein function and may reflect ongoing immune evasion [8,9]. However, these studies are limited by small numbers of isolates or biased sampling. In order to explore the genetic diversity of antigen 38 kDa in China, we used strains from 2010 National Epidemiologic Survey for TB in China, amplified the gene of the antigen 38 kDa, compared the sequences and analyzed the characteristic of mutant strains.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethics Committee of the National

Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. All patients involved in the study provided written informed consent.

2.2. Strains and DNA preparation

The spoligotype patterns of all 335 strains were showed in Fig. 1. Strains were cultured using the standard Löwenstein-Jensen medium method, heat inactivated and then used directly in polymerase chain reactions (PCRs).

2.3. PCR

The nucleotide sequences of the primers (from the 5' to 3' end) used in this study were designed by DNASTAR software according to H37Rv genome sequence and as follows: ACCACCGAGAAGATCGAAGA and AAGAAATGCAACCCGTTGAG.

The PCR were performed in a total volume of 20 µl. The PCR mixture contained 10 µl PCR buffer, 100 nM each primer, 200 µM each of the four dNTPs and 0.5U DNA Taq Polymerase (Takara). An initial denaturation of 5 min at 94 °C was followed by 35 cycles of denaturation at 94 °C for 45s, annealing at 62 °C for 45s and extension at 72 °C for 1min, followed by a final extension at 72 °C for 10min.

Negative controls (reagents only, no DNA) were included each time when the PCR was performed. The positive control was 500 pg DNA from *M. tuberculosis* H37Rv. The presence and size of each PCR product

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Fig. 1. Spoligotypes of 335 strains of *M. tuberculosis*.

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