Development of a multiplex molecular method for identification of extensively drug resistant *Mycobacterium tuberculosis* by padlock probes

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, is generally treated by a regimen of antibiotics. Resistance to the first-line antibiotics (isoniazid and rifampicin) is called multi-drug-resistant TB (MDR-TB) while resistance to most of the anti-TB drugs is called extensively-drug-resistant TB (XDR-TB). Culture (i.e., growing MTB on different antibiotic plates) is a gold standard for diagnosis of drug-resistant TB. However, one major limitation here is the long turnaround time (weeks to months) due to the slow growth of the bacteria. For rapid diagnosis of drug resistant TB, we have tested padlock probes that could detect the mutations associated with the anti-TB drugs resistance.

A padlock probe is a linear oligonucleotide comprising two target-specific sequences at both ends and a linker segment containing sequences for amplification and identification. The probe is designed so as to bring its two ends into juxtaposition upon hybridization to the target sequence. This conformation allows the probe to be circularized with great target specificity by a DNA ligase. Signal amplification can be achieved by rolling circle amplification (RCA) of the circularized padlock probes. The concatemers of DNA circles produced by RCA can then be digested, ligated into new circles and subjected to another round of RCA, known as a circle-to-circle amplification (C2CA). Quantification of RCA products (RCPs) is then performed using a single-molecule detection (SMD) approach where oligonucleotides coupled with fluorescent molecules are hybridized to the product, then pumped through a micro channel and visualized using a confocal microscope.

Mutations in the *rpoB*, *inhA*, *katG*, *gyrA* and *rrs* genes of *M. tuberculosis* make it extensively resistant to anti-TB drugs. We designed padlock probes to detect the most common mutations associated with these genes, as well as their wild type sequences. The efficiency of the padlock probes in recognizing their corresponding targets was evaluated using C2CA and SMD approaches. The specificity of the padlock probes was further evaluated to ensure that the padlock probes do not have any unspecific target recognition. At the end, we have obtained padlock probes that could efficiently and specifically recognize most of the common mutations that cause the extensive TB drug resistance.

We found, however, that a few padlock probes are to be re-designed to improve their efficiency in recognizing their corresponding targets. The next stage in the developing the padlock diagnostic is to test its multiplexibility (different padlock probes are tested at a time simultaneously on its respective target) using a Luminex assay. Further, based on the results of the multiplexibility experiments, a low density array with immobilized oligonucleotides capable of recognizing RCPs will be produced and finally tested.

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