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The design and application of DNA chips for early detection of SARS-CoV from clinical samples

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Abstract

Background: SARS coronavirus has been identified as the cause of severe acute respiratory syndrome (SARS). Few tests allow confirmation or exclusion of SARS within the first few days of infection. A gene chip is a useful tool for the study of microbial infections mainly for its capability of performing multi-target analysis in a single test.

Objectives: Investigate the possibility of early detection of SARS virus from clinical samples using the gene chip-based method.

Study design: We purified RNA from SARS-CoV obtained from routinely collected peripheral blood and sputum samples of 34 patients who had been identified as probable SARS patients by following the interim U.S. case definition. Four segments of the SARS-CoV were amplified using reverse transcription-nested PCR and the products examined using the 70-mer gene chips for SARS-CoV detection.

Results: A blind-test of both peripheral blood and sputum specimens lead to the positive detection of SARS-CoV in 31 out of 34 patients. SARS-CoV was not found in peripheral blood or sputum specimens from three patients. Two of the 34 patients were only 3 days post-onset of symptoms and were subsequently confirmed to be SARS positive. Our results indicate that the gene chip-based molecular test is specific for SARS-CoV and allows early detection of patients with SARS with detection rate about 8% higher than the single PCR test when the sputum sample is available.

Keywords: SARS; Coronavirus; SARS-CoV; Early detection; Gene chip

Abbreviations: SARS-CoV, severe acute respiratory syndrome-coronavirus; HEX, hexa-chloro-6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine

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1. Introduction

Severe acute respiratory syndrome (SARS) is a newly emerged disease associated with pneumonia in infected patients (World Health and Organization, 2003). From November 2002 to June 3, 2003, SARS virus infected 8398 people and caused 772 deaths worldwide (www.who.int/csr/sars/country/2003-06-03/en/, accessed July 2003). The genome of the SARS-CoV has been sequenced (Marra et al., 2003; Ruan et al., 2003). The earlier that a case of SARS virus is detected, the less chance there is that a patient will spread the disease to others. Various serological and molecular tests have been developed for the detection of this virus or to confirm infection. These methods include enzyme-linked immunosorbent assay (ELISA) (Ksiazek et al., 2003), indirect immunofluorescent assay (IFA) and reverse transcription (RT) followed by a real-time PCR using either the TaqMan technique or the intercalating fluorescent dye approach (Drosten et al., 2003; Peiris et al., 2003). As yet, few tests allow confirmation or exclusion of SARS within the first few days of infection. Serological methods cannot close the window period, and are not useful for early detection. Real-time PCR can only detect one target sequence a time, which means that false negative results are possible. The genome of SARS-CoV is a positive ssRNA, which means that, the mRNA sequence of SARS-CoV is same as the genome sequence, hence the copy number of SARS-CoV can not be calculated directly from the quantitative result of real-time PCR. Gene chip provide a useful tool to study microbial infections (Cheng and Kricka, 2001; Wang et al., 2002; Tao et al., 2002) mainly for its capability of performing multi-targets analysis in only one test. Recently, we have reported the use of gene chips for the detection of pathogenic bacteria and viruses in clinically relevant samples or changes in gene expression among drug-treated and untreated microbes (Cheng et al., 1998; Tao et al., 2003; Zhang et al., 2002). In this paper, we report a method for early detection of the SARS virus in clinical specimens from SARS patients. The method combines a convenient sample preparation method, nucleic acid amplification using a modified nested PCR, and a gene chip array with probes selected to cover the representative regions of the SARS-CoV genome.

2. Materials and methods

2.1. Selection of clinical specimens and control samples

Peripheral blood and sputum samples were collected from 34 patients suffering from SARS in Tsinghua University Jiuxianqiao Hospital. These patients were identified as probable SARS patients by following the interim U.S. case definition. The probable SARS case definition is based on clinical criteria and epidemiologic linkage to other SARS cases with radiographic evidence. The negative controls include 100 peripheral blood samples and 100 sputum samples that were collected from university students at Tsinghua who were determined to be free of infection based on radiological and temperature measurements. In the following experiment one negative blood control sample or sputum sample was included with 17 clinical blood samples or sputum samples during the sample pretreatment and extraction stages as a control to monitor any occurrence of contamination that may appear during the sample treatment process. In addition, one blank control using water as template was included with the 17 RT-PCR reactions on clinical samples to control the PCR procedure.

2.2. Design and construction of the gene chip

The sequence data of SARS-CoV were obtained from the curated database in GenBank. The unique and conserved regions of SARS-CoV were selected by align the released SARS-CoV sequences with each other and the latest non-redundant nucleic acid sequence database of NCBI (ftp://ftp.ncbi.nih.gov). To enable the early detection of SARS-CoV, multiple regions from open reading frame (orf) replicase 1a, spike glycoprotein and nucleocapsid protein (Rota et al., 2003) were selected as the target for hybridization. To minimize cross-hybridization, oligonucleotides of 70mer were designed following the rule from http://www.westburg.nl/download/arrayposter.pdf. To increase the immobilization efficiency of the probes, 10 thymidines were added to the 5′ end of each probe.

After an initial screening test, a set of four oligonucleotides (Sangon, Shanghai, China) were chose as the probes for identifying SARS-CoV, see Fig. 1 (a). Additionally, a set of probes for control purposes was also included. The QC probe was used to confirm the efficiency of the attachment chemistry on the surface of the substrate. For all tests this probe should always generate a strong and consistent fluorescence signal. The IC probe was designed to guarantee the sample processing procedure and the entire nested RT-PCR process operates as expected. The EC probe was used to monitor the efficiency of the hybridization process and also as a reference for quantifying the amplicons' hybridization signal. The BC was DMSO spotted on the substrate to ensure no signal detected on these spots indicating to guarantee no carry-over of the previously spotted samples.

The probes were suspended in 50% dimethyl sulfoxide (DMSO) at a concentration of 10 μM and printed on glass slides modified with amino groups (AminoSlide^{TM}, CapitalBio Corp., Beijing, China). The 6×6 arrays was printed according to the spotting pattern (Fig. 1 (b)) in duplicate on each slide. The sequence information of the probes is listed in Table 1. Concerning the current gene chip designing there are fifteen possible combinations for the occurrence of SARS-CoV positive signals. As long as one of the fifteen combinations shown in Fig. 1 (c) is detected the presence of SARS-CoV can be then ensured.