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Short communication

Resequencing microarray for detection of human adenoviruses in patients with conjunctivitis

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ABSTRACT

Background: Although high-density resequencing microarray is useful for detection and tracking the evolution of viruses associated with respiratory tract infections, no report on using this technology for the detection of viruses in patients with conjunctivitis is available.
Objectives: To test if high-density resequencing microarray can be applied to detection of viruses in conjunctival swabs for patients with conjunctivitis.
Study design: In this prospective proof-of-concept study, every 4 or 5 bacterial culture-negative conjunctival swab samples were pooled and subject to viral detection using TessArray™ Resequencing Pathogen Microarrays-Flu 3.1 (RPM-Flu-3.1). Results were compared with human adenovirus (HAdV) hexon gene PCR sequencing and viral culture.
Results: Thirty-two of the 38 conjunctival swab samples were bacterial culture-negative. Four of the 7 pooled samples were positive for HAdV using RPM-Flu-3.1. Hexon gene PCR sequencing on the 38 original individual samples showed that 3 and 4 samples contained HAdVs species D and B respectively. All the 6 samples that were positive for hexon gene PCR but negative for bacterial culture were also positive by the resequencing microarray. Viral culture was positive for HAdV type 3 in 1 sample, which was also positive by PCR and resequencing microarray.
Conclusions: Resequencing microarray is as sensitive as PCR for detection of HAdV in conjunctival swabs. Unlike viral culture and hexon gene PCR sequencing, resequencing microarray was not able to differentiate the type and species of HAdV. Development of microarrays for conjunctivitis can be performed for rapid diagnosis of the viral cause of conjunctivitis.

1. Background

Determining the microbial cause of infectious diseases usually requires detection of multiple possible viruses in clinical specimens. Isolation and electron microscopic examination are labor-intensive, time-consuming and associated with long turn-around-time. Although direct antigen detection is cost-effective with high clinical impact in respiratory infections, no comprehensive set of monoclonal antibodies against each possible virus is available for different types of clinical specimens.[1] Nucleic acid detection can potentially overcome the above problems, as designing primers for individual viruses is not difficult given the publicly available gene databases and the whole detection process can be performed within a few hours. Strategies such as multiplex PCR have been devised to detect nucleic acids of multiple viruses simultaneously. One technology that had emerged in recent years to achieve this purpose is high-density resequencing microarray platform. In this technology, total nucleic acid

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Table 1
Characteristics of patients in the present study.

Patient no.	Sex/age	Microarray pool no.	Adenovirus PCR	Viral culture	Bacterial culture	Remarks
1	M/57	–	–	–	+	
2	F/84	1	–	–	–	
3	M/69	1	–	–	–	
4	M/14	–	+	–	+	Brother of Patient 5
5	M/11	1	+	–	–	Brother of Patient 4
6	F/56	1	+	–	–	
7	M/23	1	–	–	–	
8	F/48	2	–	–	–	
9	M/9	2	–	–	–	
10	F/36	–	–	–	+	
11	F/63	2	–	–	–	
12	M/38	2	–	–	–	
13	M/15	3	–	–	–	
14	M/24	3	+	–	–	
15	M/12	3	–	–	–	
16	M/18	3	–	–	–	Patient also had keratitis
17	M/37	4	–	–	–	
18	F/20	4	–	–	–	
19	M/10	4	–	–	–	
20	F/34	–	–	–	+	
21	M/35	4	–	–	–	
22	M/58	4	–	–	–	
23	F/55	5	–	–	–	
24	M/15	5	–	–	–	Had contact history with a patient with conjunctivitis
25	M/11	5	–	–	–	
26	M/24	5	+	–	–	
27	M/54	–	–	–	+	
28	M/30	5	+	–	–	
29	F/8	6	–	–	–	
30	F/23	6	–	–	–	
31	F/8	6	–	–	–	
32	M/43	6	–	–	–	
33	F/17	6	–	–	–	
34	M/37	7	–	–	–	
35	F/9	–	–	–	+	
36	F/12	7	+	+	–	Had contact history with a patient with conjunctivitis
37	M/22	7	–	–	–	
38	M/44	7	–	–	–	

extracted from samples is subject to reverse transcription and multiplex PCR. The PCR products are pooled, purified, fragmented, end-labeled with biotin, hybridized to complementary sequences of potential targets on a microarray, and detected with fluorescent dye. The array is scanned and analyzed. It has been shown that such high-density resequencing microarray is useful for detection and tracking the evolution of viruses associated with respiratory infections.[2–5]

Infective conjunctivitis is most commonly caused by viruses and bacteria. Human adenoviruses (HAdVs) and picornaviruses are the 2 most important groups of viruses associated with conjunctivitis. Although it is associated with less mortality and morbidity compared to respiratory infections, conjunctivitis can be associated with serious outcomes, such as blindness.[6] No report on using such high-density resequencing microarray platform for detection of viruses in conjunctival swabs obtained from patients with conjunctivitis is available. In this proof-of-concept prospective study, we examined the use of one of these platforms, TessArray™ Resequencing Pathogen Microarrays-Flu 3.1 (RPM-Flu-3.1; TessArae, LLC, Potomac Falls, VA), originally designed for detection of viruses associated with respiratory infections, to detect viruses in conjunctival swabs collected from patients with conjunctivitis.

2. Objectives

To test if high-density resequencing microarray can be applied to viral detection in conjunctival swabs for patients with conjunctivitis in a prospective proof-of-concept study.

Table 2
Resequencing microarray best match results using TessArae server and NCBI blast service.

Pool no.	Patient sample no.	Best match using TessArae server	Best match using NCBI blast service	PCR sequencing of hexon gene
1	2, 3, 5, 6, 7	Human adenovirus 53 (species D) ($E = 3e^{-70}$)	Human adenovirus 37, 19 (species D) ($E = 5e^{-10}$)	Species B (Patients 4 and 5), species D (Patient 6)
2	8, 9, 11, 12	–	–	–
3	13, 14, 15, 16	–	Human adenovirus 5 (species C) ($E = 4e^{-15}$)	Species D (Patient 14)
4	17, 18, 19, 21, 22	–	–	–
5	23, 24, 25, 26, 28	Human adenovirus 53 (species D) ($E = 5e^{-92}$)	Human adenovirus 37, 8 (species D) ($E = 1e^{-18}$)	Species B (Patient 26), species D (Patient 28)
6	29, 30, 31, 32, 33	–	–	–
7	34, 36, 37, 38	Human adenovirus B strain Guangzhou (species B) ($E = 2e^{-174}$)	Human adenovirus B strain Guangzhou02 (species B) ($E = 0$)	Species B (Patient 36)

3. Study design

3.1. Patients and microbiological methods

The study protocol was reviewed and approved by the Hospital Ethics Committee. All patients who attended the Accident and Emergency Department of Tuen Mun Hospital with conjunctivitis during a 7-month period (March–September 2008) were included. In each patient with conjunctivitis, 3 conjunctival swabs were collected by rubbing the palpebral conjunctiva of the infected eye firmly with sterile saline moistened sterile swab. One was suspended in virus transport medium for total nucleic acid and DNA extraction respectively. The other 2 were used for bacterial culture and viral culture using HEp-2C, RD, MRC-5 and Vero cells.

3.2. Resequencing microarray analysis

Every 4 or 5 bacterial culture-negative samples were pooled (Tables 1 and 2) and subject to total nucleic acid extraction and resequencing microarray analysis according to manufacturer's instructions.

3.3. HAdV PCR and DNA sequencing

As the results of resequencing microarray showed that some samples contained HAdVs, DNA was extracted from all 38 individual samples and HAdV PCR and DNA sequencing performed.[7]

4. Results

4.1. Patients

Thirty-eight patients with conjunctivitis attended the Accident and Emergency Department of Tuen Mun Hospital during the 7-month period (Table 1). The male-to-female ratio was 12:7, with a median age of 24.

4.2. Resequencing microarray analysis

For the 32 bacterial culture-negative samples, every 4 or 5 samples were pooled and subject to resequencing microarray analysis (Table 2). Four pooled samples were positive for HAdV (Table 2), with no other viruses detected.

4.3. HAdV PCR and sequencing

PCR for a 189-bp fragment in the HAdV hexon gene was positive in 7 samples (Table 1). Sequencing results showed that the sequences were clustered into 3 groups. The first group (Patients 6, 14 and 28) was clustered with hexon gene sequences of species D HAdVs, whereas the second (Patients 4, 5 and 26) and third (Patient 36) groups were clustered with hexon gene sequences of species B HAdVs. The sequence from Patient 36 was most closely clustered with that of HAdV type 3.

4.4. Viral culture

HAdV type 3 was positive from the sample obtained from Patient 36. No other viruses were recovered from the other 37 patients.

5. Discussion

As there is no available resequencing microarray including all common viruses associated with acute conjunctivitis, we used the resequencing microarray for respiratory pathogens in this prospective proof-of-concept study to test if this technology can be applied to viral detection in conjunctival swabs for patients with conjunctivitis. In this study, all the 6 samples positive for HAdV PCR and tested with resequencing microarray were also positive by resequencing microarray. This showed that resequencing microarray is as sensitive as PCR. However, the RPM-Flu-3.1 microarray used in this study did not include all viruses associated with conjunctivitis. For example, coxsackie virus A24 and enterovirus 70 that were commonly associated with acute hemorrhagic conjunctivitis were not included. Development of microarrays for conjunctivitis can be performed for rapid diagnosis of the viral cause of conjunctivitis.

Despite its good sensitivity, unlike viral culture and subsequent serotyping and PCR sequencing of hexon gene, resequencing microarray was not able to differentiate the type and species of HAdV respectively. In this study, all 7 strains of HAdV detected belonged to species B or D, as shown by hexon gene sequencing. This is in line with other epidemiological studies, which have shown that HAdVs species B and D are causes of pharyngoconjunctival fever and epidemic keratoconjunctivitis.[8] The only culture-positive virus was of type 3, concurring with the results of hexon gene sequencing. Although the panel of RPM-Flu-3.1 included HAdVs type 2 (species C), 4 (species E), 7 (species B) and 17 (species D), it was not able to accurately determine the HAdV in the clinical samples to the species level. For the 2 pooled samples (pool nos. 3 and 7) with only 1 conjunctival swab positive for HAdV as determined by PCR sequencing, the species was accurately determined only in pool 7 by the panel of RPM-Flu-3.1 (Table 2). On the other hand, the non-specificity may have enabled picking up of HAdVs types not included in the panel of RPM-Flu-3.1. For example, HAdV type 3 is not included in RPM-Flu-3.1, but was successfully detected by resequencing microarray in this study.

Using resequencing microarray for screening and subsequent targeted amplification of suspected viruses saved labor. Resequencing microarray has the advantage of simultaneous detection of multiple possible microbiological causes of specific infectious disease syndromes. For example, for respiratory infections, molecular detection of viruses requires setting up PCR/RT-PCR for influenza viruses, parainfluenza viruses 1–4, HAdVs, respiratory syncytial virus, human metapneumovirus, human coronaviruses 229E, OC43, NL63 and HKU1, and rhinoviruses, which is labor-intensive and time-consuming. Although antigen detection by immunofluorescence has allowed detection of multiple viruses, monoclonal antibodies against all possible viruses are not available.[1] On the other hand, resequencing microarray allows detection of all these viruses simultaneously. In the present study, we have also shown that samples can be pooled for the resequencing microarray without sacrificing sensitivity, and subsequent PCR/RT-PCR using specific primers targeting the positive virus was performed for delineating the positive sample(s) in the pooled sample, as well as determining the species of the corresponding HAdV. The cost of using pooled samples as performed in this study was just about one quarter of that if each individual sample was studied by RPM-Flu-3.1. This has the additional advantage of saving cost, as the cost for a microarray is still high at the moment.

Conflict of interest

None declared.

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