Respiratory viruses and novel methods for virus discovery

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Summary

Viral infections represent the majority of emerging infectious diseases in humans. The etiology of a substantial part of human diseases remains unknown and it is likely that a fair percentage of these diseases are caused by unknown viruses. Therefore, identification of new viruses and improvement of the methods to discover yet-unknown viruses are important. Nowadays, application of molecular methods such as high throuput sequencing in virus discovery becomes more frequent (e.g. VIDISCA-454). These technologies are able to provide ten thousands or even millions of sequences from a clinical sample, facilitating exploration of new viral agents and virus evolution. However, cellular background signals hamper the detection of new viruses, and the determination of clinical relevance of new viral pathogens remains a significant challenge. Furthermore, with the high sensitivity of these methods, contamination of laboratories and reagents should be considered carefully. To this end, virus discovery techniques should be optimized to not only detect new viruses in clinical samples but also provide preliminary data about infectivity and pathogenesis of novel viruses.

In chapter 2, the first full-length genome sequence of clinical isolates of human coronavirus 229E was characterized and compared with the laboratory-adapted strain VR-740. The latter strain was isolated in 1962 and the two clinical strains were isolated recently in 2009 and 2010. By alignment of the nucleotide and amino acid sequences and a phylogenetic analysis we detected several intriguing differences between the clinical isolates and the laboratory-adapted strain. Changes were most frequent in the genes encoding the spike, the nucleocapsid and non-structural protein 3 and the 3'UTR. The most significant change in genome organization in the lab-adapted strain VR-740: The clinical isolates had an intact ORF4 where the lab adapted strain had a truncated ORF4.

In chapter 3, VIDISCA-454 was applied on serum samples of injecting drug users in a search for unknown viruses in HIV-patients. This resulted in the identification of a new torque teno mini virus (TTMV) genotype. The virus is chronically present in two HIV-1 infected patients, and both suffered from a pneumococcal pneumonia during follow up with extremely low B-cell counts. The direct effect of TTMV infection on these clinical features remains unknown.

Attributing the presence of a virus in patient material to a disease in that patient remains a challenge. We therefore developed an optimized virus discovery technique: antibody capture VIDISCA-454 (chapter 4). This method facilitates the identification of viruses that have provoked an

immune response in those patients. A decrease in the cellular background signal, enrichment of immunogenic virus and detection of viruses in an identity independent manner are some benefits of the method. The utility was confirmed in a pilot study using 19 clinical samples that contain respiratory and gastrointestinal viruses. In 18 of the 19 patients, viral reads from the immunogenic viruses were enriched by antibody capture. The sensitivity of antibody capture VIDISCA-454 on clinical samples was determined in chapter 5 using a clinical sample containing human rhinovirus A. The sensitivity of antibody capture VIDISCA-454 was 7.5E+04 copies/ml, only a bit reduced compared to the sensitivity of traditional VIDISCA-454 (1.5E+04 copies/ml). However, at higher concentration the percentage viral sequences in antibody capture material is higher. In chapter 6, the discovery of human rhinovirus C type 54 by the antibody capture method is described showing the utility of the antibody capture VIDISCA-454 to discover novel viruses.

In chapter 7, a universal cell culture system for respiratory viruses, human airway epithelium cell culture, is combined with VIDISCA-454 to create a virus discovery tool with the added possibility to provide information about infectiousness and cell tropism of new viruses. The efficiency of the method is verified by testing three clinical samples with influenzavirus A, influenzavirus B or human coronavirus OC43. The sequence reads covered a large part of the genome (more than 50%). The study also showed, for the first time, that influenzavirus B can be cultured in the *ex vivo* respiratory culture system by replicating in the ciliated cells.

Samenvatting

Virussen veroorzaken regelmatig nieuwe infectieziekten, bovendien bestaat het vermoeden dat onbekende virussen betrokken kunnen zijn bij een aantal ziektes waarvan de oorzaak onbekend is. Het is daarom belangrijk dat de methodologie om deze virussen op te sporen en te identificeren wordt verbeterd. De toepassing van moleculaire technieken, zoals 'high throughput' DNA en RNA sequentie-bepalingen, vindt tegenwoordig steeds meer plaats (bv. de VIDISCA-454 methode). Deze technieken kunnen tienduizenden tot miljoenen sequenties opleveren uit een klinisch monster en maken het mogelijk om inzicht te verkrijgen in nieuwe virussoorten en hun evolutie. Een probleem hierbij is de aanwezigheid van cellulair DNA en RNA in die monsters, wat de detectie van nieuwe virussen bemoeilijkt. Bovendien is het vaststellen van de klinische relevantie van een nieuw virus een serieuze uitdaging. Door de enorme gevoeligheid van deze nieuwe technieken kunnen DNA/RNA contaminaties op laboratoria en in reagentia leiden tot vals positieve uitslagen. Om deze redenen is het belangrijk dat de technieken om virussen op te sporen zodanig worden geoptimaliseerd dat ze niet alleen nieuwe virussen kunnen opsporen, maar tevens inzicht geven in de door deze virussen veroorzaakte infectie en het bijbehorende ziektebeeld.

In hoofdstuk 2 worden de eerste twee complete genoom sequenties van klinische isolaten van het humaan coronavirus 229E beschreven en vergeleken met die van de aan het laboratorium-aangepaste stam VR-740. Deze laatste stam werd geïsoleerd in 1962, terwijl de twee klinische isolaten recentelijk (in 2009 en 2010) circuleerden. Door de nucleïnezuur- en eiwit-sequenties te vergelijken en de fylogenetische verwantschap te bepalen zijn er een aantal opvallende verschillen ontdekt tussen de klinische isolaten enerzijds en de laboratorium-aangepaste stam anderzijds. Vooral de genen die coderen voor het zogenaamde 'spike' eiwit, het nucleocapside eiwit, het niet-structurele eiwit NSP-3, en de niet-coderende 3'UTR vertonen verschillen. De meest in het oog springende verandering bij de klinische isolaten is de aanwezighied van een intact open leesraam 4, welke is onderbroken in de laboratorium stam.

In hoofdstuk 3 staat beschreven hoe VIDISCA-454 wordt toegepast op serum monsters van hiv-geïnfecteerde druggebruikers om te zoeken naar onbekende virussen in deze immuun-gecompromitteerde individuen. Dit resulteerde in de identificatie van een nieuw genotype van torque teno mini virus (TTMV). Dit virus bleek chronisch aanwezig in twee patiënten, waarbij het opvallend is dat beide patiënten een longontsteking doormaakten die werd veroorzaakt door de

pneumokok bacterie. Bovendien hadden beide patiënten een extreem lage hoeveelheid B-cellen. Het directe effect van de TTMV infectie op het klinische beeld bij deze patiënten blijft vooralsnog onbekend.

Het blijft lastig om te bepalen in hoeverre de aanwezigheid van een virus daadwerkelijk bijdraagt aan een ziektebeeld. Een verbeterde methode om virussen te detecteren en direct te koppelen aan een bepaald ziektebeeld wordt beschreven in hoofdstuk 4: De antilichaam-gekoppelde VIDISCA-454. Deze methode maakt het mogelijk om selectief virussen te identificeren die in patiënten een immuunreactie hebben opgewekt. De voordelen van deze techniek zijn velerlei, waaronder verlaging van de cellulaire DNA en RNA achtergrond en verrijking van het immunogene virus. Door deze meer selectieve aanpak wordt het ook makkelijker om virussen te vinden die weinig tot geen gelijkenis vertonen met thans bekende virussen. De nieuwe methode werd getest op 19 patiënten monsters met reeds gediagnosticeerde respiratoire en gastro-intestinale virussen. In 18 van de 19 patiënten werden meer virale sequenties gevonden met behulp van de antilichaam-gekoppelde VIDISCA-454 dan met de reguliere VIDISCA-454 methode. De gevoeligheid van de antilichaam-gekoppelde VIDISCA-454 wordt beschreven in hoofdstuk 5 door gebruik te maken van een klinisch monster met rhinovirus A. Deze gevoeligheid is ongeveer 7.5 E+04 virusdeeltjes per milliliter, iets minder gevoelig dan de traditionele VIDISCA-454 (1.5E+04 virusdeeltjes per mL), maar bij hogere concentraties virus is het percentage virus sequenties sterk verrijkt na antilichaam koppeling. In hoofdstuk 6 wordt de nieuwe methode toegepast voor de ontdekking van een nieuw type rhinovirus (type 54).

In hoofdstuk 7 wordt beschreven hoe het celkweek systeem van humaan luchtweg epitheel gecombineerd kan worden met VIDISCA-454 om een virus opsporing methode te creëren die tevens informatie kan verschaffen over het cel tropisme van een nieuw virus. De efficiëntie van deze methode is getest op 3 klinische monsters die influenzavirus A, influenzavirus B of humaan coronavirus OC43 bevatten. De aan de celkweek gekoppelde VIDISCA-454 sequentie informatie leverde een groot deel van de sequentie van het virale genoom (meer dan 50%). Tevens laat deze studie zien dat influenzavirus B gekweekt kan worden in het *ex-vivo* respiratoir kweeksysteem en - voor het eerst - dat influenzavirus B voornamelijk cellen met trilharen infecteert.

A word of thanks

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Curriculum vitae

Seyed Mohammad Jazaeri Farsani was born on November 20th 1977 in Farsan, Iran. He finished high school and afterward he went to the Shahrekord University and graduated in 1998 in veterinary medicine. Then he continued his education at Bahonar University of Kerman and he obtained his Bachelor in laboratory sciences in 2001. In 2003 he became interested in virology and he studied virology at the Faculty of Medical Sciences of the Tarbiat Modares University in Tehran, where he worked on DNA vaccines of herpes simplex viruses. He received his master degree in 2006. In 2010 he moved to the Netherlands and started his PhD research in the group of Dr. Lia van der Hoek at the Laboratory of Experimental Virology at the Academic Medical Center of the University of Amsterdam, headed by professor Ben Berkhout. His PhD project mainly focused on respiratory viruses and novel methods for virus discovery. The results of this research are described in this thesis.

AMC Graduate School for Medical Sciences

PhD Portfolio

Name PhD student: Seyed Mohammad Jazaeri Farsani

PhD period: June 2010- October 2014

Name PhD supervisor: Dr. Lia van der Hoek and Prof. dr. Ben Berkhout

PhD training

General courses	Year	ECTS
- AMC World of Science	2010	1
- Basic Laboratory Safety	2011	0.4
- Oral presentation in English	2013	0.8
- Scientific Writing in English for Publication	2012	1.5
- Systematic Reviews	2013	0.7
- Crash course	2011	0.4
- Management of Medical Literature	2010	0.5

Specific courses

-	Infectious Diseases	2010	1.3
-	Advanced Immunology	2013	2.9
-	DNA Technology	2011	2.1
-	Basic Microscopy Course	2012	1.6
-	Bioinformatics	2011	1.1
-	Mass Spectrometry, Proteomics and Protein Research	2011	2.1
-	Gene Targeting and Transgenic Strategies	2013	0.4
-	Advanced qPCR	2014	0.5
-	Course in virology (Erasmus MC)	2014	1
-	One health course (Bonn MC)	2014	3.5

Seminars, workshops and master classes

-	Roche-454 sequencing workshop	2013	1
-	Metagenomics in Virology	2013	1
-	Science and business models in emerging infectious diseases	2013	1
-	Weekly department seminars	2010-2014	4
-	Weekly PhD student meeting	2010-2014	4
-	Refereeravond (department seminars)	2010-2012	1

Tutoring, Mentoring

-	Guiding student internship	2013	2

List of publications

(1)   **Jazaeri Farsani SM**, Dijkman R, Jebbink MF, Goossens H, Ieven M, Deijs M, et al. The first complete genome sequences of clinical isolates of human coronavirus 229E. Virus Genes 2012 Dec;45(3):433-9.

(2)   **Jazaeri Farsani SM**, Jebbink MF, Deijs M, Canuti M, van Dort KA, Bakker M, et al. Identification of a new genotype of Torque Teno Mini virus. Virol J 2013 Oct 30;10(1):323.

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