Infection, Genetics and Evolution 44 (2016) 403–411

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Strong down-regulation of glycophorin genes: A host defense mechanism against rotavirus infection

Antonio Salas [a,b,*], Guillermo Marco-Puche [c], Juan Carlos Triviño [c], Alberto Gómez-Carballa [a,b], Miriam Cebey-López [b], Irene Rivero-Calle [b,d], Lucía Vilanova-Trillo [b,d], Carmen Rodríguez-Tenreiro [b,d], José Gómez-Rial [b,d], Federico Martinón-Torres [b,d]

[a] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, and GENPOB Research Group, Instituto de Investigaciones Sanitarias (IDIS), Hospital Clínico Universitario de Santiago, Galicia, Spain
[b] Grupo de Investigación en Genética, Vacunas, Infecciones y Pediatría (GENVIP), Hospital Clínico Universitario, Universidade de Santiago de Compostela (USC), Galicia, Spain,
[c] Sistemas Genómicos, Paterna, Valencia, Spain
[d] Translational Pediatrics and Infectious Diseases, Department of Pediatrics, Hospital Clínico Universitario de Santiago de Compostela, Galicia, Spain

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 29 July 2016
Accepted 30 July 2016
Available online 02 August 2016

Keywords:
Rotavirus
Transcriptome
RNA-Seq
Cell cycle
Glycophorin

ABSTRACT

The mechanisms of rotavirus (RV) infection have been analyzed from different angles but the way in which RV modifies the transcriptome of the host is still unknown. Whole transcriptome shotgun sequencing of peripheral blood samples was used to reveal patterns of expression from the genome of RV-infected patients. RV provokes global changes in the transcriptome of infected cells, involving an over-expression of genes involved in cell cycle and chromatin condensation. While interferon *IFI27* was hyper-activated, interferon type II was not suggesting that RV has developed mechanisms to evade the innate response by host cells after virus infection. Most interesting was the inhibition of genes of the glycophorins A and B (*GYPA/B*) family, which are the major sialoglycoproteins of the human erythrocyte membrane and receptor of several viruses for host invasion. RV infection induces a complex and global response in the host. The strong inhibition of glycophorins suggests a novel defense mechanism of the host to prevent viral infection, inhibiting the expression of receptors used by the virus for infection. The present results add further support to the systemic nature of RV infection.

1. Introduction

Rotaviruses (RV) are the leading cause of severe, dehydrating diarrhea in children under five years of age worldwide (Parashar et al., 2003). It has been estimated that approximately 453,000 children, each year, die from diarrheal disease caused by RV, with most of these cases occurring in developing countries (Tate and Parashar, 2011), and about two million requiring hospitalization (Simpson et al., 2007). Most deaths attributable to RV infection occur in countries where there is limited access to the available RV vaccines (Patton, 2012). However, immunity to RV is not completely understood.

A universal response of the host cell to viral infection is the secretion of cytokines belonging to the interferon family. Activation of the interferon system is an extremely powerful antiviral mechanism that directly prevents replication of viruses within infected cells. Interferon type II or IFN-γ (also known as IFN gamma) produced inactivated host T-cells and natural killer cells are particularly involved in the regulation of the immune and inflammatory responses. Therefore, a successful replication of viruses in the host organism involves controlling innate antiviral responses. One of the different mechanisms used by viruses is to interfere with antiviral responses in the infected cell by preventing detection of viral components. It has been reported that the suppression of the interferon response could be mediated by a number of viral mechanisms including sequestration of viral RNA in viruplasms and dsRNA in virus particles (Sherry, 2009). These mechanisms aim to alter the function of transcription factors that initiate antiviral responses (Arnold et al., 2013). According to Sherry (2009) (Sherry, 2009) mammalian reoviruses and RVs have evolved specific strategies to evade the type I interferon (IFNs) antiviral mechanisms. Consequently, RV-infected cells are generally characterized by low levels of interferon expression (Arnold et al., 2013; Randall and Goodbourn, 2008).

On the other hand, it has been recently reported that RV antigenemia and viremia are commonly detected in children hospitalized for RV gastroenteritis and may be associated with increased severity of fever and vomiting (Hemming et al., 2014; Moon et al., 2012; Rivero-Calle et al., 2016).

Virus interactions with the host occur at various levels in all stages of replication. Interactions are important for RV colonization, and the ability of the virus to enable the host to recognize the presence of the RV is

* Corresponding author at: Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain.
E-mail address: antonio.salas@usc.es (A. Salas).

http://dx.doi.org/10.1016/j.meegid.2016.07.044
1567-1348/

Table 1
Clinical characteristics of the samples analyzed in the present study. All patients and controls were sampled in Galicia (Spain) and were of European ancestry.

Sample ID2	Disease status	Gender	Prematurity	Relevant clinical features	Hospital admission (days)	Fever	Vesikari score	Clinical score
GLO_41	Control	Male	No	–	–	–	–	–
GLO_13	Control	Male	No	–	–	–	–	–
GLO_17	Control	Female	No	Admitted for suspected sepsis at birth	–	–	–	–
GLO_66	Control	Male	No	–	–	–	–	–
GLO_76	Control	Female	No	–	–	–	–	–
GLO_81	Control	Male	No	–	–	–	–	–
GLO_84	Control	Female	No	–	–	–	–	–
GLO_85	Control	Male	No	–	–	–	–	–
GLO_86	Control	Female	No	–	–	–	–	–
GLO_91	Control	Female	No	Gastroesophageal reflux, admitted suspected sepsis at birth	–	–	–	–
GLO_94	Control	Female	No	–	–	–	–	–
GLO_98	Control	Male	No	–	–	–	–	–
GLO_99	Control	Male	No	–	–	–	–	–
GLO_2	Patient	Female	No	Failure to thrive since 6 months of age	4	Yes	10	Moderate
GLO_4	Patient	Female	No	–	4	Yes	11	Moderate
GLO_5	Patient	Female	No	Admitted at birth due to perinatal respiratory distress	1	No	11	Moderate
GLO_6	Patient	Female	No	Hyperbilirubinemia and jaundice; required phototherapy	3	Yes	12	Moderate
GLO_100	Patient	Female	Yes (28 weeks)	Admitted at birth for prematurity, respiratory distress and apneas	5	Yes	15	Severe

crucial. Only a few studies have investigated gene expression patterns in RV-infected patients (Arnold et al., 2013). At the same time, in very recent years, the analysis of the complete transcriptome of different organisms using massive sequencing procedures has acquired great importance in the study of global patterns of gene expression. Next generation sequencing (NGS) is capable of sequencing, in parallel and massively, millions of cDNA fragments in a single sequencing process rapidly and relatively inexpensively. Massive sequencing techniques of total RNA (RNA-Seq) offer the opportunity to obtain global information of transcriptomic status of a specific tissue, or even a single cell, not only providing information about gene expression levels but also allowing the identification of alternative splicing events, unknown transcripts, processes of gene fusion or identification of mutations simultaneously.

To the best of our knowledge, no previous study has aimed to understand how the transcriptome of the host is altered by RV infection globally. The present project involved the analysis of the full transcriptome using RNA-Seq of patients infected with RV versus control individuals.

2. Material and methods

2.1. Samples

Infected pediatric cases ($n = 6$) and controls ($n = 13$) were prospectively recruited at the Hospital Clínico Universitario of Santiago de Compostela (Galicia; Spain) during the period 2013 to 2014. Patients hospitalized with acute gastroenteritis were included when a positive RV antigen was detected in stool. RV-infected patients were ethnically

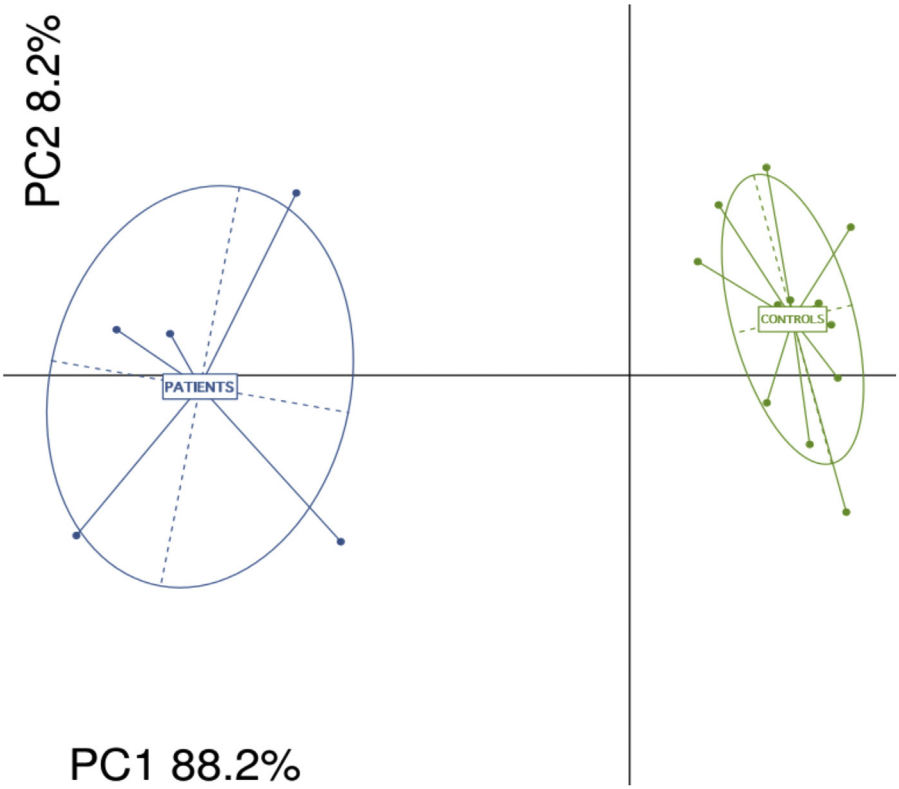

Fig. 1. PCA analysis of whole transcriptome expression patterns in RV-infected patients and controls.