Virology 485 (2015) 305–312

Contents lists available at ScienceDirect

Virology

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

Transcriptome sequencing and development of an expression microarray platform for liver infection in adenovirus type 5-infected Syrian golden hamsters

Baoling Ying, Karoly Toth, Jacqueline F. Spencer, Rajeev Aurora, William S.M. Wold *

Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, 1100 S. Grand Boulevard, St. Louis, MO 63104, United States

ARTICLE INFO

Article history:
Received 3 June 2015
Returned to author for revisions
3 July 2015
Accepted 30 July 2015

Keywords:
Adenovirus
Transcriptome
Syrian hamster
Microarray
Liver
Antiviral response
Innate immunity

ABSTRACT

The Syrian golden hamster is an attractive animal for research on infectious diseases and other diseases. We report here the sequencing, assembly, and annotation of the Syrian hamster transcriptome. We include transcripts from ten pooled tissues from a naïve hamster and one stimulated with lipopolysaccharide. Our data set identified 42,707 non-redundant transcripts, representing 34,191 unique genes. Based on the transcriptome data, we generated a custom microarray and used this new platform to investigate the transcriptional response in the Syrian hamster liver following intravenous adenovirus type 5 (Ad5) infection. We found that Ad5 infection caused a massive change in regulation of liver transcripts, with robust up-regulation of genes involved in the antiviral response, indicating that the innate immune response functions in the host defense against Ad5 infection of the liver. The data and novel platforms developed in this study will facilitate further development of this important animal model.

Introduction

The Syrian golden hamster has been used broadly in biomedical research for decades, especially as a model for cancer development. More recently, it has been served as models in research on diabetes (Bhathena et al., 2011), atherosclerosis (Dillard et al., 2010; Jove et al., 2013), and neural plasticity (Staffend and Meisel, 2012). Also, the Syrian hamster is frequently employed in studies of infectious disease, as it is highly susceptible to infection with a wide range of viruses, bacteria, and parasites (Espitia et al., 2010; Zivcec et al., 2011). The Syrian hamster has been used to study emerging and highly pathogenic RNA viral infections and virus-induced diseases, including flaviviruses (West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, Yellow fever virus), alphaviruses (Venezuelan-, Western-, Eastern equine encephalitis virus), henipavirus (Nipah virus), arenaviruses (Pirital virus, Pichinde virus), bunyaviruses (Rift valley fever virus, Punta Toro virus, Andes virus), filoviruses (Ebola virus, Marburg virus), and SARS-coronavirus (Ebihara et al., 2013; Gowen and Holbrook, 2008; Holbrook and Gowen, 2008; Nakayama and Saijo, 2013; Roberts et al., 2005). Further, the Syrian hamster has proved to be a useful model to evaluate vaccines and anti-viral drugs against both RNA and DNA viruses (Julander et al., 2007, 2011; Monath et al., 2010; Morrey et al., 2004, 2008; Toth et al., 2008; Wold and Toth, 2012).

Still further, the Syrian hamster has served as a model to study human adenovirus (Ad) pathogenesis and to evaluate oncolytic Ad vectors in cancer gene therapy (Bortolanza et al., 2007; Cerullo et al., 2012; Dhar et al., 2009a, 2009b, 2012, 2014; Lichtenstein et al., 2009; Spencer et al., 2009; Thomas et al., 2006, 2007, 2008; Toth et al., 2007; Wold and Toth, 2012; Ying et al., 2009a; Young et al., 2013a, 2013b). Human Ads are double-stranded DNA viruses with a non-enveloped icosahedral capsid. There are approximately 60 types (previously referred to as serotypes) that are classified into seven species from A to G (Berk, 2013). In immunocompetent healthy individuals, most Ad infections are generally mild and self-limiting, resulting in infections in the upper respiratory tract, gastrointestinal, and urinary tracts (Wold and Ison, 2013). Some serotypes can cause serious ocular illness such as epidemic keratoconjunctinitis. In immunocompromised individuals, especially in pediatric hematopoietic stem cell transplant patients, Ads can cause severe disseminated multi-organ infections which are sometimes lethal (Echavarria, 2008; Ison, 2006; Lion, 2014; Matthes-Martin et al., 2012, 2013; Sandkovsky et al., 2014; Stercz et al., 2012; Wold and Ison, 2013).

Syrian hamsters are permissive for human Ads (reviewed in Wold and Toth (2012)). Ad5 infection following intravenous (i.v.) administration results in the hamster's body weight loss, elevated serum liver enzyme levels, and virus replication in the liver, lungs, and other

* Corresponding author.

http://dx.doi.org/10.1016/j.virol.2015.07.024
0042-6822/

organs (Lichtenstein et al., 2009; Ying et al., 2009). When the hamster is immunosuppressed using high-dose cyclophosphamide, Ad5 replication continues for extended periods in multiple organs, especially the liver, resulting in increased mortality (Thomas et al., 2008; Toth et al., 2008). This extended replication in hamsters recapitulates human Ad infection in immunocompromised patients.

A major limitation for using the Syrian hamster as an animal model for infectious disease is the lack of information on the hamster genome and its transcription. The Syrian hamster genome has just been sequenced and released but has not been annotated (NCBI Bio Project 77669). The transcriptome of Chinese hamster ovary and Syrian baby hamster kidney cell lines as well as hamster brain and liver tissue have been analyzed (Johnson et al., 2014; Schmucki et al., 2013; Vishwanathan et al., 2015). More recently, the Syrian hamster transcriptome with additional tissues from brain, lung, spleen, kidney, heart, and liver has been published (Tchitchek et al., 2014), but the complete global transcriptome representing whole hamster transcripts, especially genes engaging in immune responses, has not been documented. Quantitative reverse transcriptase PCR (qRT-PCR) to detect and quantify some cytokines and chemokines has been published, but to our knowledge, microarrays specific to global hamster genes have not been developed due to the insufficiency of genetic data.

We report here the sequencing, assembly, and annotation of the Syrian hamster whole transcriptome, together with the generation of hamster microarrays. By using these new platforms, we describe the first global transcriptional analysis of gene expression in the liver of Ad5-infected Syrian hamsters. Our results provide unique insights into acute Ad5 infection of the liver, an organ often infected in disseminated Ad disease in humans.

Results

Hamster transcriptome sequencing and annotation

Our goal was to produce a comprehensive catalog of transcripts expressed in the adult hamster. To this end, we isolated RNA from 10 organs from a naïve animal and a hamster treated with LPS. The organs were chosen because they are the targets of many viruses (e.g. liver) or these organs are complex (e.g. brain) in that they are composed of many cells types and thus express many genes. LPS treatment was included to increase the transcription of genes involved in immune responses, as would be seen in microbiological infections. Pooled RNA was subjected to library construction for deep sequencing.

Illumina HiSeq sequencing produced 5.1×10^{10} raw 100 bp paired-end reads (Table 1). Raw Illumina RNA-Seq data were adapter trimmed, validated, and filtered based on quality. Sequences that passed validation and quality checks were assembled via the *de novo* RNA-seq assembler Trinity (Grabherr et al., 2011; Haas et al., 2013). Trinity assembled contigs were validated via ESTscan and custom software, resulting in a set of 49,176 high-confidence transcripts. These high-confidence transcripts denote the assembled transcriptome. Of the 49,176 transcripts in the assembled transcriptome, 44,705 sequences (91%) had at least one BLASTP hit, and 30,956 (63%) had at least one InterProScan hit. The raw reads, as well as the assembled transcripts, have been deposited in GenBank under BioProject ID PRJNA285471.

Generation of custom hamster microarray and analysis

To assess the genome-wide response to Ad5 infections of the liver, two groups of hamsters were administered i.v. with either 10^{10} pfu of Ad5 or PBS (mock-infection). Ad5 replicates preferentially in the liver of Syrian hamsters following i.v. administration (Ying et al., 2009). Liver RNAs were extracted 18 h post-infection (p.i.) and gene expression was analyzed by a custom designed microarray. Two biological replicates (independent infections) were performed and each replicate was analyzed in triplicate (Ad5-infected was labeled with Cy3 and mock was labeled with Cy5). A dye-swap experiment was also performed to correct for any dye bias, for a total of four technical replicates per biological replicate (one slide). The data were analyzed using an ANOVA model as previously described (Churchill, 2004; Kiesel et al., 2007).

Table 1
Analysis of transcriptome by RNA-seq.

A. Tissue sampled	B. Transcriptome	
Blood	Total raw read (100bp paired end)	5.1×10^{10}
Bone Marrow	Total number of validated	49,176
Brain	Transcripts	
Kidney	Total number of unique transcripts	42,707
Liver	Total number of unique genes	34,191
Cervical lymph node		
Lung		
Muscle		
Spleen		
Thymus		

A. Each tissue was sampled from two hamsters; one treated with LPS and the second was untreated. B. Pooled RNA from both hamsters was sequenced on Illumina HiSeq platform. Summary statistics for raw reads, number of validated transcripts, unique transcript (eliminating transcripts with alternative start site or 5′ end) and total number of genes (based on BLASTP to rat genome).

A total of 7110 genes in the liver were differentially expressed with a $p \leq 0.01$. Of these, 3788 were upregulated and 3322 were down-regulated in response to Ad5 infection. All significantly differentially expressed genes were further subjected to Gene Ontology analysis (GO) to identify functional categories of change in gene expression. As shown in Tables 2A and 2B, transcripts in a number of critical function categories are over-represented. Most notably, genes involved in immune response (57 out of 64 genes (57/64)), defense response to virus (16/17), and antigen processing and presentation (27/31) were largely upregulated in the liver, although some were downregulated (Table 2A). Also largely upregulated were genes associated with regulating DNA-dependent transcription (82/119), signal transduction (76/109), cell adhesion (18/18), and cell communication (16/21) (Table 2A). In contrast, when the most down-regulated genes were investigated for GO function, there was over-representation of genes associated with oxidation–reduction process (214/249), metabolic process (82/101), carbohydrate metabolic process (45/69), DNA topological change (16/21), protein translation (15/18), and tricarboxylic acid cycle (7/7) (Table 2B). Differentially regulated genes are also overrepresented in the GO categories of peptidoglycan catabolic process and transmembrane transport. In the GO category of peptidoglycan catabolic process, 4 genes were upregulated and 4 genes were downregulated (p value = 1.17e − 9). In the GO category of transmembrane transport, 11 genes were upregulated and 12 genes were downregulated (p value = 1.10e − 08)

Table 3 shows a more detailed depiction of differentially expressed genes in the GO category of anti-viral responses: 16 out of 17 genes were upregulated, including toll-like receptor 7 and 2, IL-6, and interferon-inducible proteins such as OAS family members and PKR.

Validation of microarray data by qRT-PCR

To validate the differential gene expression profiles obtained by microarray analysis, qRT-PCR was performed. First, we confirmed the suitability of four housekeeping genes, namely PPI, RPS6KB1, HBP2, and GAPDH. These genes range from low, medium–low, medium, and