Chinese Chemical Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

An RNA polymerase I-driven human respiratory syncytial virus minigenome as a tool for quantifying virus titers and screening antiviral drug

Yuan-Hui Fu [1], Ya-Ru Liu [1], Yan-Peng Zheng, Nan Jiang, Yue-Ying-Jiao, Wei Li, Xiang-Lei Peng, Jin-Sheng He *

College of Life Sciences and Bioengineering, Beijing Jiaotong University, Beijing 100044, China

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 15 May 2016
Accepted 26 May 2016
Available online xxx

Keywords:
Human respiratory syncytial virus
Gaussia luciferase
Minigenome
Antiviral drug
Drug-screening

ABSTRACT

Human respiratory syncytial virus (RSV) is an important pediatric pathogen of lower respiratory tract worldwide. No vaccines and antiviral drugs are available. Herein the use of an RNA polymerase I-driven RSV minigenome for analyzing RSV replication and screening anti-RSV drugs was investigated. The RNA polymerase I (Pol I) was used to transcribe RSV minigenome from the constructed plasmid, designated pHM-RSV-Gluc, of minigenome cDNA which comprised trailer region, gene start sequence (GS), reverse complementary copy of Gaussia luciferase (Gluc) gene, gene end sequence (GE), and leader region in the direction of 5′–3′ end and was flanked by promoter and terminator of Pol I. The expression of Gluc was confirmed in pHM-RSV-Gluc transfected HEp-2 cells following RSV infection and had the characteristics of dose-dependent, which provided a rapid, sensitive, and quantitative method for quantifying virus titers and screening antiviral drugs.

1. Introduction

Human respiratory syncytial virus (RSV) is the most important cause of lower respiratory tract diseases during infants and children under two years of age worldwide [1,2], which has caused serious socioeconomic burden [3]. Currently, Palivizumab and Ribavirin are the few options for RSV specific-treatment and prophylaxis. Palivizumab is restricted to infants who are at high risk to develop severe RSV infection and costs high, while the application of ribavirin is impeded by questionable efficacy and safety reasons [4]. Therefore, the development of novel anti-RSV drugs becomes very urgent.

RSV contains single-stranded, negative-sense, non-segmented RNA genomes. The gene order is as follows: 3′-Leader-NS1-NS2-N-P-M-SH-G-F-M2-1/M2-2-L-trailer-5′ [2]. Four of the viral proteins, N, P, M2-1, and L, are associated with the viral genomic RNA to form the ribonucleoprotein (RNP) complex [3]. The untranslated leader region and trailer region at the 3′ and 5′ terminals of genome, and gene end sequence (GS) as well as gene start sequence (GE) flanking the subgenomes have been shown to be necessary and sufficient cis-acting elements for viral RNA replication and transcription [4]. Consequently, RSV minigenome containing a single reporter gene as an alternative of the RSV coding genes can simulates the processes of transcription and replication of RSV genome. T7 polymerase-driven RSV minigenome had been used as a tool in quantitating RSV replication and screening anti-RSV drugs. However, T7 polymerase-driven RSV minigenome needs an exogenously introduced T7 polymerase, which potentially limits the system's efficiency because of technical difficulties in the expression of T7 in all cells. Another substantial limitation of T7 polymerase-driven RSV minigenome involves the incorporation of an ribozyme sequence of hepatitis D virus (HDV), neighboring the minigenome and at the 3′ end of the transcription cassette [5]. While the cleavage by the HDV ribozyme can produce an authentic 3′ or 5′ terminus of RSV genome, the cleavage efficiency of the transcript by HDV ribozyme is very low and therefore additional potential to limit the production of functional minigenome transcripts would occur within this system [6]. In contrast, the RNA polymerase I (Pol I) is a eukaryotic host cell polymerase which is normally localized to the nucleoli and can provide a substantial advantage in terms of the development of minigenome systems, since it alleviates the need to supply the polymerase in trans [6]. Pol I-driven paramyxovirus minigenome detection model has been

* Corresponding author.
E-mail address: jshhe@bjtu.edu.cn (J.-S. He).
[1] These authors contributed equally to the present work.

http://dx.doi.org/10.1016/j.cclet.2016.06.018
1001-8417/

successfully established and applied, and the Pol I-driven minigenome system has the same or even better signal strength compared with T7 [6,7]. Taken together, these results suggest that the Pol I-driven minigenome expression system is another effective means to analyze the replication of paramyxovirus. In this article, an RNA polymerase I-driven RSV minigenome applied for quantitating RSV replication and screening anti-RSV drugs is investigated. Pol I was used to transcribe RSV minigenome from the constructed plasmid, designated pHM-RSV-Gluc, of minigenome cDNA which comprised trailer region, GS, reverse complement copy of Gaussia luciferase (Gluc) gene, an alternative of viral genes, and GE as well as leader region in the direction of 5′–3′ end and was flanked by promoter and terminator of Pol I. After pHM-RSV-Gluc being transfected into RSV infected HEp-2 cells, (−) vRNA is generated by Pol I in cell nucleus. Then (+) mRNA is generated by RNA dependent RNA polymerase of RSV, and Gluc is expressed, which is an indicator of the replication level of RSV.

In summary, the constructed RSV minigenome under the control of Pol I can be used as a tool for quantitating RSV replication and screening anti-RSV drugs.

2. Experimental materials and methods

2.1. Cell and virus

HEp-2 (Human laryngeal carcinoma, ATCC, Rockefeller, MD, USA) cells were grown in DMEM (Gibco BRL, Gaithersburg, USA) containing 2 mmol/L L-glutamine (Amresco, Solon, USA) and 10% fetal bovine serum (FBS, Hyclone, Logan, USA). Subgroup A RSV Long strain was kindly provided by Prof. Y. Qian, Capital Institute of Pediatrics, Beijing, China. All experiments with infectious virus were performed in the BSL-2 laboratory at the Beijing Jiaotong University, Beijing, China.

2.2. Purification and titration of RSV

Subgroup A RSV Long strain was propagated in HEp-2 cells in DMEM supplemented with 2% FBS (Invitrogen), L-glutamine (2 mmol/L), penicillin G (40 U/mL), streptomycin (100 μg/mL) and 0.2% sodium bicarbonate. RSV was purified by ultracentrifugation and titrated for infectivity by immunoenzyme assay. RSV titers were expressed as plaque-forming units (pfu).

2.3. Construction of pHM-RSV-Gluc

To construct pHM-RSV-Gluc, the sequences of RSV-Gluc (shown as Fig. 1) were synthesized by BGI (Shenzhen, China). The RSV-Gluc expressing cassette with Pol I enhancer/promoter and terminator was cloned into the pHM vector to produce pHM-RSV-Gluc.

2.4. Gaussia luciferase assays

The supernatant from the transfected and infected HEp-2 cells was transferred into 96 well plate, Gluc activity was assayed by use of LUMIstar (BMG LABTECH, Ortenberg, Germany) according to the manufacturer's instructions after adding the chromogenic substrate of Gaussia Luciferase Flex Assay Kit (NEB, Ipswich, USA). The results were documented by photography and/or evaluated with OPTIMA software (BMG LABTECH, Ortenberg, Germany). The resultant signal strength was expressed as relative light unit (RLU).

2.5. Cell viability assays

Cell viability was detected by the MTS method. Assays are performed by adding MTS (Promega, Madison, USA) mixture directly to culture wells. After incubating for 4 h, the absorbance was recorded at 490 nm with an ELISA plate reader (Tecan, Männedorf, Switzerland).

2.6. Statistical analyses

Statistical analyses were performed using the SPSS 11.5 software (SPSS, Chicago, USA). Comparison of differences was conducted using the Tukey test. $P < 0.05$ was considered significant.

3. Results and discussion

3.1. The construction of pHM-RSV-Gluc and the purification of RSV

In order to ensure the stability of the experiment, the purified RSV with titers above 10^8 pfu/mL was obtained and used through

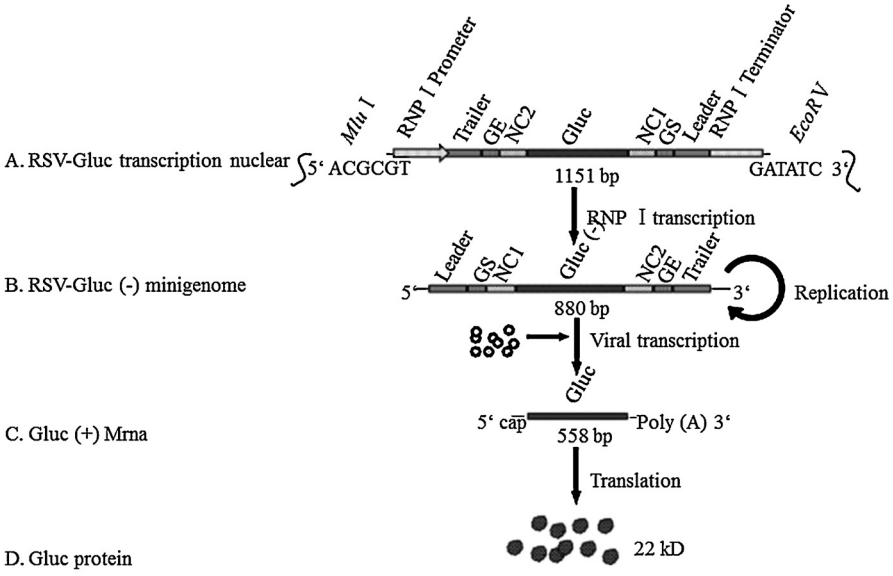

Fig. 1. Schematic representation of reporter gene expression from vRNA-oriented polymerase I-driven minigenome. vRNA-oriented polymerase I-driven minigenome contained a trailer region, GS (gene start sequence), NC1 (non-coding sequence of NS1), a reverse complement copy of Gaussia luciferase (Gluc) gene in place of viral genes, NC2 (non-coding sequence of L), GE (gene end sequence), and a leader region in the direction of 5′–3′ end and was flanked by a promoter and a terminator of RNP I.

Please cite this article in press as: Y.-H. Fu, et al., An RNA polymerase I-driven human respiratory syncytial virus minigenome as a tool for quantifying virus titers and screening antiviral drug, Chin. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.cclet.2016.06.018