Biochemical and Biophysical Research Communications 462 (2015) 426–432

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Alteration of cell cycle progression by Sindbis virus infection

Ruirong Yi [a], Kengo Saito [a], Naohisa Isegawa [b], Hiroshi Shirasawa [a, *]

[a] Department of Molecular Virology, Graduate School of Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chiba 260-8670, Japan
[b] Laboratory Animal Center, Graduate School of Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chiba 260-8670, Japan

ARTICLE INFO

Article history:
Received 11 April 2015
Available online 12 May 2015

Keywords:
Sindbis virus
Cell cycle
Viral replication

ABSTRACT

We examined the impact of Sindbis virus (SINV) infection on cell cycle progression in a cancer cell line, HeLa, and a non-cancerous cell line, Vero. Cell cycle analyses showed that SINV infection is able to alter the cell cycle progression in both HeLa and Vero cells, but differently, especially during the early stage of infection. SINV infection affected the expression of several cell cycle regulators (CDK4, CDK6, cyclin E, p21, cyclin A and cyclin B) in HeLa cells and caused HeLa cells to accumulate in S phase during the early stage of infection. Monitoring SINV replication in HeLa and Vero cells expressing cell cycle indicators revealed that SINV which infected HeLa cells during G_1 phase preferred to proliferate during S/G_2 phase, and the average time interval for viral replication was significantly shorter in both HeLa and Vero cells infected during G_1 phase than in cells infected during S/G_2 phase.

1. Introduction

Sindbis virus (SINV) is an RNA virus belonging to the *Alphavirus* genus in the *Togaviridae* virus family. SINV is transmitted to birds and mammals by mosquito bites and subsequently spreads throughout the body through the bloodstream [1]. SINV infection induces no or only mild symptoms (fever, rash, and arthralgia) in humans [2]. SINV has the potential to induce apoptosis in infected mammalian cells, but establishes a non-cytolytic persistent infection in arthropod cells [2,3]. In addition, the 67-kDa, high-affinity laminin receptor has been identified as a surface attachment factor that mediates SINV infection of mammalian cells [4], and is highly expressed in various human cancers [5,6].

We reported previously that SINV has oncolytic features and demonstrates antitumoral effects in various cancers, including cervical and ovarian cancer [7], and human oral squamous carcinoma cells [8]. Replication-defective SINV vectors have also been developed to target and eradicate tumors [9,10].

As an oncolytic virus, the favorable features of SINV include rapid production of high-titer virus, efficient infection of a variety of cancer cells, and a high RNA replication rate in the cytoplasm [11]. The preferable characteristics of SINV for cancer therapy might be attributed to the combination of favorable viral growth and the uncontrolled cell proliferation of cancer cells, including deregulation of the cell cycle. In normal cells, cell cycle regulator proteins, cyclin-dependent protein kinases (CDKs), cyclins, and CDK inhibitory proteins regulate progression through G_1, S, G_2 and M phases in the cell cycle. Controls by these regulators are often disturbed in cancer cells, which tend to remain in cycle [12]. Many viruses can affect the cell cycle progression of host cells to favor viral replication. Regarding DNA viruses, such as simian virus 40, adenovirus, and papillomavirus, infected cells are promoted into S phase (reviewed by Ref. [13]). Several RNA viruses also reportedly affect the cell cycle (reviewed by Ref. [14]). Recently, malignant glioma cells infected with an RNA virus, alphavirus M1, were shown to accumulate in S phase by down-regulating p21 protein [15]. The interaction between SINV replication and the host cell cycle has not been studied in detail, particularly in the context of oncolysis.

In this study, we analyzed the dynamics of cell cycle phases of HeLa and Vero cells infected with SINV in order to elucidate the interaction between SINV replication and the host cell cycle.

2. Materials and methods

2.1. Cell lines

HeLa cells were obtained from the American Type Culture Collection (ATCC; Rockville, MD). Vero cells were laboratory stock. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma) with 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2.

* Corresponding author. Fax: +81 43 226 2045.
E-mail address: sirasawa@faculty.chiba-u.jp (H. Shirasawa).

http://dx.doi.org/10.1016/j.bbrc.2015.04.148

2.2. Viral stocks and construction of recombinant virus

We used the laboratory stock of the AR339 strain of SINV. The plasmid pTR339-GFP-2A was kindly provided by Dr. Hans W. Heidner (University of Texas, San Antonio, TX; [16]). The BFP gene is substituted for the GFP gene in the pTR339-GFP-2A plasmid, to yield pTR339-BFP-2A.

2.3. RNA transcription

For in vitro transcription, pTR339-BFP-2A plasmid was purified using the HiSpeed Plasmid Midi Kit (Qiagen), linearized with *Xho*I (Biolab), and transcribed in vitro with the RiboMAX™ Large-Scale RNA production system-SP6 kit (Promega).

2.4. Imaging of cultured cells

Cells were grown on a 35-mm dish in DMEM with 10% FBS. After virus infection and incubation at 37 °C in 5% CO_2 for 1.5 h, cells were subjected to long-term, time-lapse imaging using a computer-assisted fluorescence microscope (Olympus, FV10i) at 37 °C in 5% CO_2. Images were recorded every 30 min. Three filter cubes were chosen for fluorescence imaging: mKusabira-orange (excitation wavelength 548 nm, emission wavelength 559 nm) to observe Fucci orange, Azami Green (excitation wavelength 493 nm, emission wavelength 505 nm) to observe Fucci green, and Blue-Narrow (excitation wavelength at 405 nm, emission wavelength 420–460 nm) to observe BFP. We used Fluoview version 3.1 (Olympus) for image acquisition and analysis.

2.5. Western blotting

Equal volumes of extracted protein were loaded onto SDS-polyacrylamide gels (Atto Corporation), transferred onto PVDF membranes (Trans-Blot Turbo™ Transfer Pack, BIO-RAD), and analyzed with antibodies.

2.6. Cell cycle analysis

Cells were collected and washed twice with PBS. Next, cells were treated with reagents from the CycleTEST™ PLUS DNA Reagent Kit (Becton Dickinson and Company) and analyzed on a BD Accuri™ C6 Flow Cytometer (Becton Dickinson and Company) equipped with FACScan's fluorescence 2 (FL2) detector. The collected data was analyzed using FlowJo 7.6.5 (TreeStar Company).

2.7. Statistical analysis

All values were expressed as mean ± SD. Statistical analyses were performed using the software Statcel2, version 2 (OMS, Tokyo, Japan). Values of $P < 0.05$ were considered statistically significant.

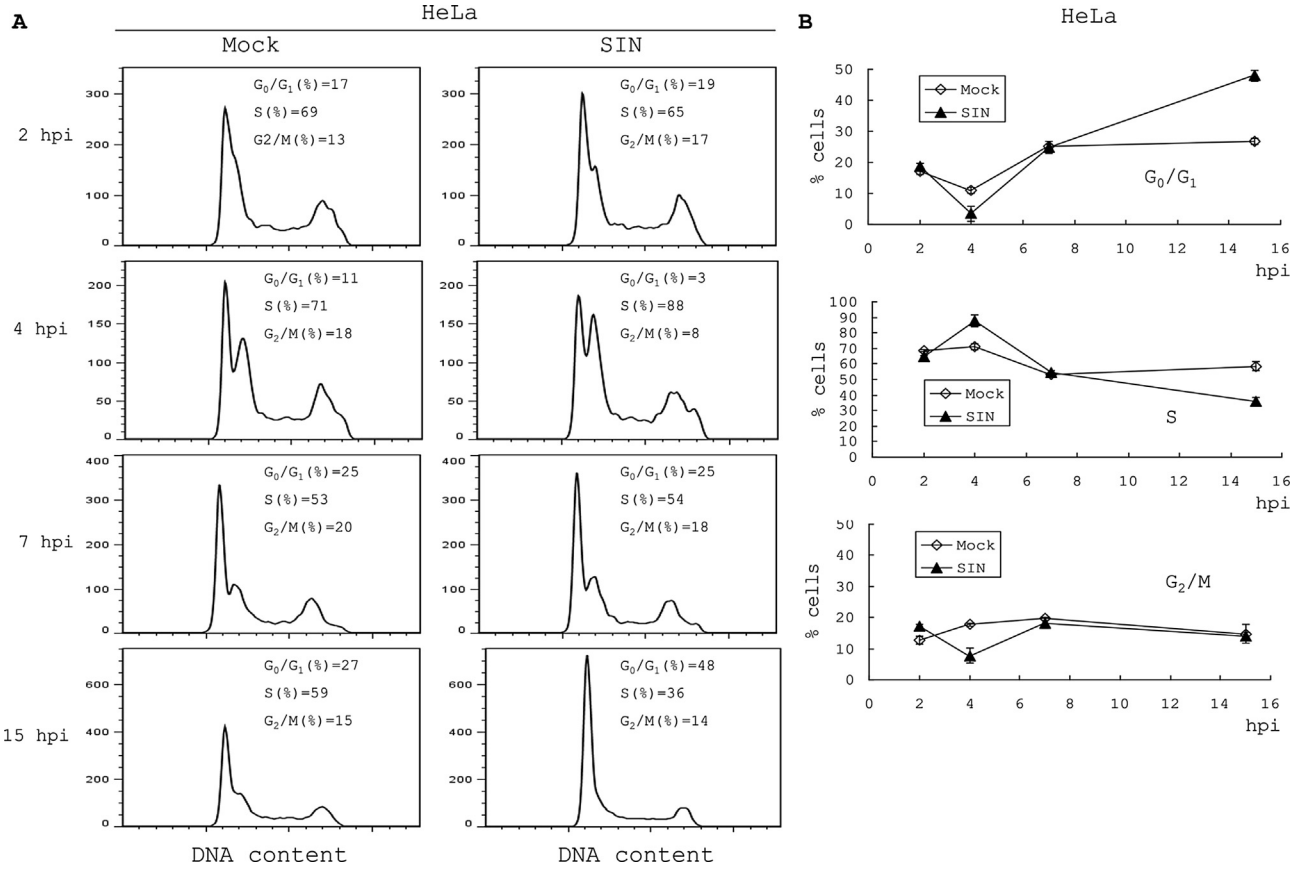

Fig. 1. The effect of SINV infection on cell cycle progression. HeLa or Vero cells were infected with 1 MOI of SINV and subjected to FACS analysis at the indicated hour post-infection (hpi). (A) FACS analysis of mock-infected (Mock) and SINV-infected (SIN) HeLa cells at 2, 4, 7, and 15 hpi. Percentages of cells in G_0/G_1, S, and G_2/M are shown. (B) The time course of proportions of mock- and SINV-infected cells in G_0/G_1, S, and G_2/M phases. (C) FACS analysis of mock-infected (Mock) and SINV-infected (SIN) Vero cells at 2, 4, 7, and 14 hpi. Percentages of cells in G_0/G_1, S, and G_2/M are shown. (D) The time course of proportions of mock- and SINV-infected Vero cells in G_0/G_1, S, and G_2/M phases. Data presented are the mean of three independent experiments. Error bars indicate standard error.