Biochemical and Biophysical Research Communications 391 (2010) 176–181

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A role for heparan sulfate in viral surfing

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ARTICLE INFO

Article history:
Received 22 October 2009
Available online 10 November 2009

Keywords:
Heparan sulfate
Viral surfing
Herpes simplex virus type-1
Filopodia
Virus
Glycoprotein
Transport

ABSTRACT

Heparan sulfate (HS) moieties on cell surfaces are known to provide attachment sites for many viruses including herpes simplex virus type-1 (HSV-1). Here, we demonstrate that cells respond to HSV-1 infection by enhancing filopodia formation. Filopodia express HS and are subsequently utilized for the transport of HSV-1 virions to cell bodies in a surfing-like phenomenon, which is facilitated by the underlying actin cytoskeleton and is regulated by transient activation of a small Rho GTPase, Cdc42. We also demonstrate that interaction between a highly conserved herpesvirus envelope glycoprotein B (gB) and HS is required for surfing. A HSV-1 mutant that lacks gB fails to surf and quantum dots conjugated with gB demonstrate surfing-like movements. Our data demonstrates a novel use of a common receptor, HS, which could also be exploited by multiple viruses and quite possibly, many additional ligands for transport along the plasma membrane.

Introduction

Heparan sulfate (HS) proteoglycans, key components of cell surfaces and extra-cellular matrix, modulate physiological activities and influence cell growth and differentiation by interacting with a variety of regulatory factors [1]. The versatile ability of HS to interact with a variety of molecules can also be exploited by pathogens including viruses to invade human cells. Herpesviruses, like many other viruses, initiate infection of the host cell by first attaching to HS moieties present on cell surfaces [2,3]. The attachment then initiates the process of viral penetration into host cells. Herpesvirus entry, exemplified by herpes simplex virus type-1 (HSV-1), requires participation from multiple viral glycoproteins (gB, gC, gD, gH, and gL) and cellular receptors [3,4]. Binding of HSV-1 to HS is mediated by gB and gC, followed by interaction of gD with one of its three receptors: HVEM, nectin-1, and 3-O-sulfated heparan sulfate or 3-OS HS [4–7]. Binding of gD to its receptor is essential for viral penetration, which ultimately results in deposition of viral DNA for replication in the nucleus [4].

Although it is well established that HS provides the initial docking sites for the virions, it is not clear where on the host cell surface this interaction occurs first. In this context, projections on the plasma membrane, such as filopodia and retraction fibers, could potentially be important. Both are thin, rod-like, inter-convertible cell surface extensions formed by bundles of parallel actin filaments [8] that grow by the assembly of actin via a process that is signaled by activation of a Rho-family GTPase, Cdc42 [9,10]. Many pathogenic viruses including human herpesviruses such as HSV-1 [11], Epstein-Barr virus [12] and human herpesvirus-8 (HHV-8) [13] also activate Cdc42 during invasion of the host cells.

While filopodia may contribute to viral spread in many instances by facilitating virus particles to bud out [13,14], they could also play a role by facilitating entry of exogenous virions. Recently a phenomenon of viral surfing, where by virions attach to filopodia/retraction fibers and travel down these extensions to reach the cell body for infection was reported for unrelated retroviruses and papillomavirus [15,16]. Here, we provide novel details about HSV-1 surfing and suggest a new role for HS as a mediator of the viral transport phenomenon. The interaction with HS, via HSV-1 gB, results in lateral viral movement along the length of filopodia to bring the virions closer to the cell body. The involvement of HS raises an intriguing possibility that all HS-binding virions may exploit their interactions with HS for targeted transport to cell bodies. It also implicates HS in ligand transport in general.

Materials and methods

Plasmids, cell lines and reagents. The plasmids used in these experiments were pPEP98 (gB), and pPEP99 (gD) [5]. Wild-type CHO-K1, A mutant CHO cell line, pgsA-745 [17] and the gB complementing cell line D6 [18] were used. CHO cells stably expressing

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doi:10.1016/j.bbrc.2009.11.027

nectin-1 were provided by P. Spear (Northwestern University, Chicago IL). Cells were grown in Ham's F12 medium supplemented with 10% fetal bovine serum (FBS) and containing geneticin (500 μg/ml) for complimenting cell lines. HEK-293 and HeLa cells were passaged with DMEM supplemented with 10% FBS while Vero cells were passaged with DMEM supplemented with 5% FBS. Antibody to heparan sulfate (10E4, U.S. biological), gB (Virusys Corporation), and gD (Abcam) were used at predetermined concentrations. Heparinase III was obtained from Sigma. Nectin-1 antibody, PRR1, (Immunotech) was used in a 1:100 dilution. Secondary HRP antibody (Jackson Immunoresearch Laboratories) was used in a 1:5000 dilution. FITC conjugated secondary anti-mouse IgG (SIGMA) was used in 1:200 dilution. Texas Red phalloidan (Invitrogen) was used at a dilution of 1:100.

Virus stocks and purification. Wild-type KOS, K26GFP and KO82-K26GFP viruses were used [18,19]. KO82-K26GFP lacks gB, and was grown in a complementing cell line (D6) that stably expresses gB. Virus was propagated on Vero cells and purified using a sucrose gradient as previously described [8,10]. The β-galactosidase-expressing recombinant HSV-1(KOS) gL86 was kindly provided by P. Spear (Northwestern University, Chicago IL). Since the virus lacks gL, it was grown in a complementing Vero cell line that stably expresses gL [6].

Imaging and analysis. All cell images were taken using a 100× oil objective (plan-apo 1.4) on a Leica SP2 laser scanning confocal microscope (Leica). Cells were grown on a 35-mm glass-bottom dishes (Mattek) coated with collagen (BD Biosciences). Before imaging at 37 °C, cells were washed with PBS, and cells were placed in DMEM/10% FBS. In some experiments, cells were serum starved for eight hours. K26GFP virus was imaged at 30 min after infection. In some experiments a high multiplicity of infection of 100–500 was used to capture particles in a given plane. For live cell imaging, brightfield and GFP channels were imaged every 30 s. Images were acquired using Leica confocal software. All videos, entire images, and analysis were processed using Metamorph (Molecular Devices), Leica confocal software, and Photoshop (Adobe).

Downregulation of Cdc42 and its effect on entry. Small interfering (si)-RNA targeting Cdc42, two different RNA duplexes (CDC42 Validated Stealth RNAi) were purchased from Invitrogen. Transfection of siRNA duplexes were performed in HeLa cells with Lipofectamine 2000 (Invitrogen) according to manufacturer's instruction, and seeded onto glass bottom dishes (Mattek). As a negative control for Cdc42 siRNA low GC RNAi Negative Control Duplexes (Invitrogen) was used. The transfection efficiency of each duplex was confirmed by Block-It Fluorescent Oligo (Invitrogen). Viral entry assay was described previously [6].

Indirect immunofluorescence. Indirect immunofluorescence assay was done on Vero and CHO-K1 cells. Cells were adhered to glass bottom dishes, and subsequently blocked in PBS with 3% (w/v) bovine serum albumin, for 1 h at room temperature. Cells were then incubated with either heparan sulfate antibody (US Biologicals), HVEM antibody, or Nectin-1 antibody (Immunotech) for 1 h at room temperature. As a negative control, primary antibody was omitted. FITC conjugated secondary anti-mouse IgG (SIGMA) was used in 1:200 dilution.

Cell lysate and quantum dot preparations. Cells were transfected in 6-well dishes with appropriate plasmid. After 24 h post transfection, cells were detached using cell dissociation buffer (Invitrogen) and sonicated on ice for 6 s intervals, and then put on ice for 6 s. This was repeated a total of six times, and the solution was passed through a 0.8 μm filter. For quantum dot experiments, HeLa cells were blocked in 6% BSA for 1 h. Anti-gB monoclonal antibody (10B7, Virusys Inc.) was incubated for 1 h with Qdot@605 goat anti-mouse IgG (Invitrogen) and the mixture was then incubated with gB for 1 h before addition to cells with a final end concentration of 0.04 μM, which were pre-blocked with 6% BSA. As a negative control, primary antibody was excluded from quantum dots.

Western blot. Cdc42 activation and western blot assay was described previously [11]. Time points for Cdc42 activation were set at 0 min, 30 s, 1 and 5 min.

Results and discussion

HSV-1 can induce filopodia

In order to examine the early morphological changes upon HSV-1 infection, a number of cell lines were examined at high magnification on a Leica SP2 laser scanning confocal microscope (Leica). As shown in Fig. 1A, significant (40–200%) increase in the number of

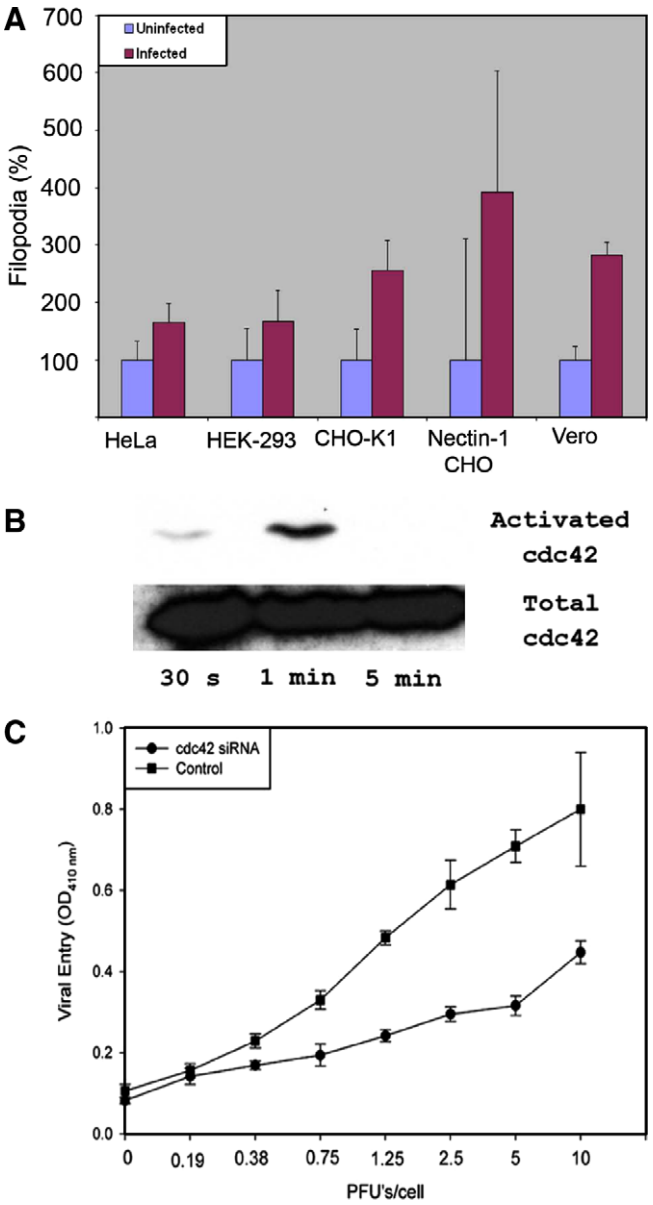

Fig. 1. Exogenous HSV-1 can induce filopodia formation. (A) Increase in the number of filopodia upon exposure to HSV-1 was counted for cells indicated. Counting was done 15–30 min before and after the addition of virus. Numbers are represented as percentages to better compare different cell lines. Three independent experiments were counted for each cell type ($n = 25$). Numbers of filopodia are presented as means with error bars showing standard deviation. (B) Western Blot analysis confirms activation of Cdc42 at the time points indicated. (C) Down-regulation of Cdc42 inhibits HSV-1 entry. HeLa cells were transfected with siRNA against Cdc42 or control siRNA and exposed to a β-galactosidase expressing HSV-1(KOS) virus.