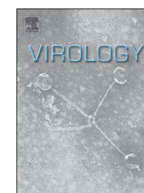
Virology 494 (2016) 78–88

Contents lists available at ScienceDirect

Virology

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



Hemagglutinin of influenza A virus binds specifically to cell surface nucleolin and plays a role in virus internalization

Che-Man Chan [a,b,c], Hin Chu [a,b,c], Anna Jinxia Zhang [a,b,c], Lai-Han Leung [e], Kong-Hung Sze [a,b,c], Richard Yi-Tsun Kao [a,b,c], Kenn Ka-Heng Chik [b], Kelvin Kai-Wang To [a,b,c], Jasper Fuk-Woo Chan [a,b,c], Honglin Chen [a,b,c], Dong-Yan Jin [d], Liang Liu [e], Kwok-Yung Yuen [a,b,c,*]

[a] *State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong, China*
[b] *Department of Microbiology, The University of Hong Kong, Hong Kong, China*
[c] *Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong, China*
[d] *Department of Biochemistry, The University of Hong Kong, Hong Kong, China*
[e] *State Key Laboratory of Quality Research in Chinese Medicine and Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Macau, China*

ARTICLE INFO

Article history:
Received 11 March 2016
Returned to author for revisions
6 April 2016
Accepted 6 April 2016

Keywords:
Influenza virus
Nucleolin
Internalization
Entry
Cell surface
VOPBA
Hemagglutinin

ABSTRACT

The hemagglutinin (HA) protein of influenza A virus initiates cell entry by binding to sialic acids on target cells. In the current study, we demonstrated that in addition to sialic acids, influenza A/Puerto Rico/8/34 H1N1 (PR8) virus HA specifically binds to cell surface nucleolin (NCL). The interaction between HA and NCL was initially revealed with virus overlay protein binding assay (VOPBA) and subsequently verified with co-immunoprecipitation. Importantly, inhibiting cell surface NCL with NCL antibody, blocking PR8 viruses with purified NCL protein, or depleting endogenous NCL with siRNA all substantially reduced influenza virus internalization. We further demonstrated that NCL was a conserved cellular factor required for the entry of multiple influenza A viruses, including H1N1, H3N2, H5N1, and H7N9. Overall, our findings identified a novel role of NCL in influenza virus life cycle and established NCL as one of the host cell surface proteins for the entry of influenza A virus.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Influenza A viruses are the most important viral respiratory disease agents that cause seasonal, pandemic, or avian influenza with significant morbidity and mortality in humans (Chan et al., 2013; Chen et al., 2013; Cheng et al., 2012; To et al., 2013, 2014; Yuen et al., 1998). Viral envelope protein hemagglutinin (HA) attaches to the sialic acid receptors on the host cell membrane and triggers the process of virus internalization through multiple endocytic pathways including predominantly clathrin-mediated endocytosis, caveolin-mediated endocytosis, and clathrin- or caveolae-independent pathways of macropinocytosis (de Vries et al., 2011; Matlin et al., 1981; Rust et al., 2004; Sieczkarski and Whittaker, 2002).

Nucleolin (NCL) is an abundant and ubiquitously expressed protein that has been implicated in a remarkably large number of cellular activities. Despite its primary localization within the nucleolus, NCL is also localized to the nucleoplasm, cytoplasm, as well as the cell surface. Importantly, NCL can function as a shuttle protein and can mediate the transportation of cargo proteins between compartments (Hovanessian et al., 2010; Srivastava and Pollard, 1999). NCL has been described to play roles in the replication and intracellular trafficking of multiple viruses. For example, NCL interacts with the US11 protein of herpes simplex virus type 1 (HSV-1) to facilitate the export of US11 from the cell nucleus to the cytoplasm (Greco et al., 2012). Notably, NCL has been identified as a functional cellular receptor for the entry of the human respiratory syncytial virus (RSV) (Tayyari et al., 2011). In the context of influenza viruses, NCL has been demonstrated to interact with the NS1 protein of H3N2 and results in the nucleolar retention of the NS1 protein (Melen et al., 2012; Murayama et al., 2007). NCL is also a receptor for TNF-α inducing protein of *Helicobacter pylori*, and is implicated in the carcinogenesis of *H. pylori* associated gastric carcinoma (Fujiki et al., 2014).

In the present study, we identified that the cell surface NCL served as an additional cell surface molecule that was required for

* Correspondence to: Carol Yu Centre for Infection, Department of Microbiology, The University of Hong Kong, Room 423, University Pathology Building, Queen Mary Hospital, Hong Kong, China.
E-mail address: kyyuen@hku.hk (K.-Y. Yuen).

http://dx.doi.org/10.1016/j.virol.2016.04.008
0042-6822/© 2016 Elsevier Inc. All rights reserved.

the efficient internalization of human influenza A H1N1 PR8 virus in human lung epithelial cells, A549. Using the virus overlay protein binding assay (VOPBA), our data suggested that the HA of PR8 virus bound specifically to NCL during virus internalization. In line with this finding, blocking A549 with a NCL antibody or blocking H1N1 virus with purified NCL protein reduced cellular entry of the H1N1 virus. Perhaps most importantly, we further demonstrated that siRNA depletion of endogenous NCL downregulated the efficiency of clathrin-mediated endocytosis and significantly diminished the entry of the H1N1 virus. Moreover, we assessed the dependency of NCL on the entry of additional influenza A viruses including H3N2, H5N1, and H7N9. Our data suggested that NCL is required for the entry of not only H1N1, but for viruses from different influenza A subtypes. Overall, our study identified a novel role of NCL in the influenza virus life cycle and established NCL as a crucial host cell surface protein for the entry of influenza A virus.

2. Results

2.1. The influenza PR8 virus bound to cell surface nucleolin (NCL)

VOPBA was employed to identify potential novel cell surface membrane proteins that influenza virus PR8 bound in A549 cells. Surface proteins were selectively biotinylated by membrane impermeable biotins. Biotin-labeled surface extracts were then enriched by binding to avidin agarose and separated from non-biotinylated cellular proteins. Eluted surface proteins and unbound flow-through were subsequently evaluated by Western blot against streptavidin-HRP conjugate, calreticulin (an endoplasmic reticulum marker) and pan-cadherin (a plasma membrane marker). As shown in Fig. 1, most proteins in the eluted fractions reacted with streptavidin-HRP conjugate (Fig. 1A, eluted) and only a small amount of proteins were found in the flow-through (Fig. 1A, unbound). In contrast to the eluted extracts that were enriched with

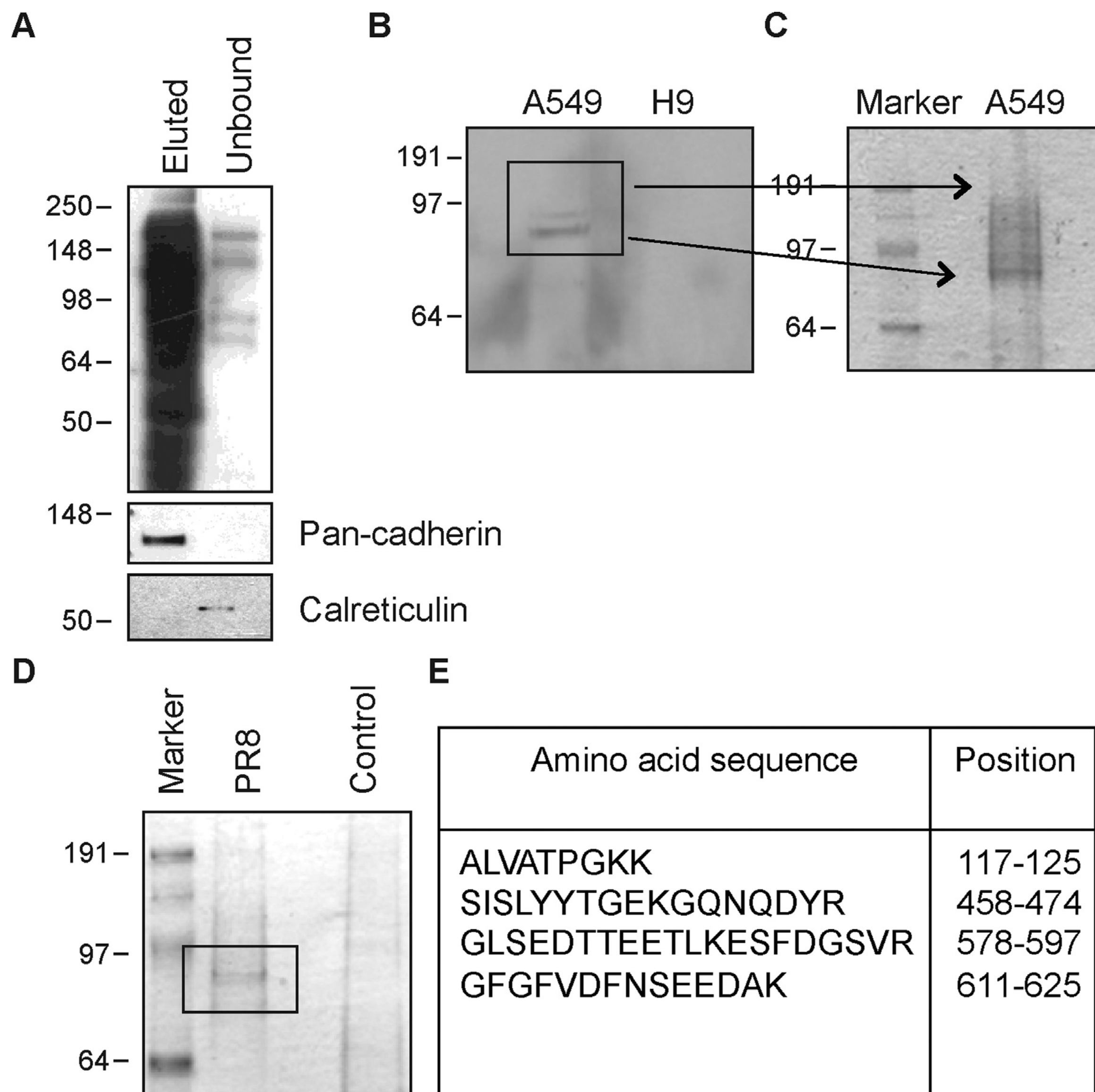


Fig. 1. Identification of NCL in biotinylated cell membrane protein fraction bound by influenza A H1N1 PR8. Eluted biotinylated surface extracts from avidin agarose were run on SDS PAGE and subjected to Western blot analysis with streptavidin-HRP conjugate. Eluted biotinylated surface extracts (A, Eluted) and unbound flow-through (A, Unbound) were tested by Western blotting with a plasma membrane marker, pan-cadherin, and an endoplasmic reticulum marker, calreticulin. VOPBA of A549 surface extracts with PR8 (B, A549). A human non-permissive line H9 was included as a control (B, H9). The relevant gel fraction (B, boxed) was cut, electro-eluted, dialyzed and separated on 4–12% gradient gel (C). A 90 kDa fragment was pulled down from the purified fraction by PR8 cross-linked onto NHS activated sepharose. The silver-stained gel band (D, boxed) was cut and sent for protein identification by mass spectrometry. A control pull down was performed with sepharose without cross-linking with PR8 (D, Control). Tryptic peptides were mapped and the 90 kDa protein was identified as NCL. Major tryptic peptides covering the protein are shown (E).

Download English Version:

https://daneshyari.com/en/article/6138444

Download Persian Version:

https://daneshyari.com/article/6138444

Daneshyari.com