Proteomic Analysis of Membrane Proteins of Vero Cells: Exploration of Potential Proteins Responsible for Virus Entry

Donghua Guo, Qinghe Zhu, Hong Zhang, and Dongbo Sun

Vero cells are highly susceptible to many viruses in humans and animals, and its membrane proteins (MPs) are responsible for virus entry. In our study, the MP proteome of the Vero cells was investigated using a shotgun LC-MS/MS approach. Six hundred twenty-seven proteins, including a total of 1839 peptides, were identified in MP samples of the Vero cells. In 627 proteins, 307 proteins (48.96%) were annotated in terms of biological process of gene ontology (GO) categories; 356 proteins (56.78%) were annotated in terms of molecular function of GO categories; 414 proteins (66.03%) were annotated in terms of cellular components of GO categories. Of 627 identified proteins, seventeen proteins had been revealed to be virus receptor proteins. The resulting protein lists and highlighted proteins may provide valuable information to increase understanding of virus infection of Vero cells.

Introduction

THE VERO LINEAGE was isolated from kidney epithelial cells extracted from an African green monkey (Yasumura and Kawakita, 1963). African green monkey kidney (Vero) cells have been proven to be highly susceptible to many viruses in humans and animals, such as coronaviruses, and are widely used for the primary isolation and cultivation of the Vero-infected viruses and novel viruses (Ozaki *et al.*, 2004; Pan *et al.*, 2012; Zhou *et al.*, 2012; Cheng *et al.*, 2013; Vlecken *et al.*, 2013). The virus entry-associated proteins on the surface of target cells are important basic information for designing the prevention and treatment strategies of the viral diseases. At present, there is no detailed information for addressing virus entry-associated proteins in Vero cells.

The cellular membrane proteins (MPs) are considered as main targets during virus infections, and the information regarding the MPs could provide some basis for the understanding of virus entry and infection (Jolly and Sattentau, 2013). Currently, proteomics techniques are an effective tool for characterization of protein profiles (Yim *et al.*, 2004; Sun *et al.*, 2013). In our study, the shotgun proteomics measure was employed to investigate the MP profiling of Vero cells. Our aim is to provide some valuable information for further understanding of virus entry-associated proteins in Vero cells.

Materials and Methods

Extraction of MPs of Vero cells

The Vero cell line was obtained from the American Type Culture Collection (ATCC), and was stored in liquid nitrogen

at the Division of Swine Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences. The Vero cells were cultured in the DMEM supplemented with 10% fetal bovine serum and were incubated at 37°C in an atmosphere containing 5% CO₂. Approximately 6×10^6 Vero cells were used for extraction of the MPs according to the manufacturer's instructions of Membrane Protein Extraction kit (BestBio Company). The extracted MPs of Vero cells were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using silver staining. The extracted MPs of Vero cells were stored at –80°C.

In-solution digestion

The MP sample of Vero cells was centrifuged at 14,000 *g* for 45 min and the protein content in the supernatant was quantified by the Bradford method using the Bio-Rad protein assay reagent. Protein digestion was performed according to the filter-aided sample preparation (FASP) procedure described by Wiśniewski *et al.* (2009). Briefly, 3 µL of MP sample of Vero cells (28.62 µg) was solubilized in 30 µL of SDT buffer (4% SDS, 100 mM DTT, 150 mM Tris-HCl pH 8.0) at 90°C for 5 min. The detergent, DTT, and other low molecular weight components were removed using 200 µL of UA buffer (8 M Urea, 150 mM Tris-HCl pH 8.0) by repeated ultrafiltration (Microcon units, 30 kDa). Then, 100 µL of 0.05 M iodoacetamide in the UA buffer was added to block reduced cysteine residues and the samples were incubated for 20 min in darkness. The filter was washed with 100 µL of UA buffer three times and then 100 µL of 25 mM NH₄HCO₃ twice. Finally, the protein suspension was digested with 2 µg

trypsin (Promega) in 40 μ L of 25 mM NH_4HCO_3 overnight at 37°C, and the resulting peptides were collected as a filtrate.

LC-MS/MS

Experiments were performed on a Q-Exactive mass spectrometer that was coupled to Easy nLC (Thermo Fisher Scientific). The peptide mixture was loaded onto a C18 reversed phase column (15 cm long, 75 μ m inner diameter) packed in-house with RP-C18 5 μ m resin in buffer A (0.1% formic acid in HPLC-grade water) and separated with a linear gradient of buffer B (0.1% formic acid in 84% acetonitrile) at a flow rate of 250 nL/min controlled by IntelliFlow technology over 60 min. MS data were acquired using a data-dependent top10 method dynamically choosing the most abundant precursor ions from the survey scan (300–1800 m/z) for HCD fragmentation. Determination of the target value is based on predictive Automatic Gain Control (pAGC). Dynamic exclusion duration was 20 s. Survey scans were acquired at a resolution of 70,000 at m/z 200 and resolution for HCD spectra was set to 17,500 at m/z 200. Normalized collision energy was 27 eV and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum fill time, was defined as 0.1%. The instrument was run with peptide recognition mode enabled.

Protein identification and analysis

MS/MS spectra were searched using Maxquant 1.3.0.5 against Uniprot database (Homo.sapiens, 133549 sequence, download March 1, 2013). For protein identification, the following options were used. Peptide mass tolerance=20 ppm, MS/MS tolerance=6 ppm, enzyme=trypsin, missed cleavage=2, fixed modification: carbamidomethyl (C), variable modification: oxidation(M), peptides FDR $\leq$ 0.01; protein FDR $\leq$ 0.01. Gene ontology (GO) categories of the identified proteins were performed with the DAVID webtool available at the website <http://david.abcc.ncifcrf.gov/> according to the protein uniprot accession numbers (Huang *et al.*, 2009a, 2009b). Virus receptor proteins were analyzed based on the current reports.

Results

Identification and analysis of MPs of the Vero cells

SDS-PAGE with silver staining indicated that the MPs were successfully extracted from Vero cells, and its concentration was 9.54 μ g/ μ L (Fig. 1). After in-solution digestion using the FASP method, the MP sample of Vero cells was identified using the shotgun LC-MS/MS proteomics technique, and further analysis was performed by GO annotation. A total of 1839 peptides were identified in MP samples of the Vero cells, including 627 proteins; In 627 proteins, 10 proteins (1.59%) are putative or uncharacterized proteins, 307 proteins (48.96%) were annotated in terms of biological process of GO categories, 356 proteins (56.78%) were annotated in terms of molecular function of GO categories, and 414 proteins (66.03%) were annotated in terms of cellular components of GO categories (Table 1). The raw data are shown in Supplementary Tables S1-S3 (Supplementary materials are available online at <http://www.liebertpub.com/dna>). Further analysis of the GO annotation indicated that the rich biological process was the cellular process (GO:0009987)

FIG. 1. Separation of membrane proteins (MPs) of the Vero cells by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Lane M, protein marker (10–170 kDa); Lane 1, the extracted MPs from Vero cells; Lane 2, bovine serum albumin control.

accounting for 94% (289/307) (Fig. 2A); The rich molecular function was binding (GO:0005488) accounting for 90% (320/356) (Fig. 2B); The rich cellular component was membrane (GO:0016020) accounting for 79% (327/414) (Fig. 2C).

Analysis of virus receptor proteins in identified MPs of the Vero cells

The identified MPs from Vero cells were analyzed according to the current reports regarding virus receptor proteins. A total of 17 proteins have been revealed to be virus entry receptors, involving in rabies virus (RV), dengue virus

TABLE 1. THE PROTEINS IDENTIFIED FROM MEMBRANE PROTEIN SAMPLE OF VERO CELLS

	Number of proteins
Identified peptides	1839
Identified proteins	627 (100%)
Putative or uncharacterized proteins	10 (1.59%)
Annotated proteins in GO categories of biological process	307 (48.96%)
Annotated proteins in GO categories of molecular function	356 (56.78%)
Annotated proteins in GO categories of cellular component	414 (66.03%)

GO, gene ontology.

FIG. 2. Gene ontology (GO) categories of the identified MPs from Vero cells. **(A)** Biological process GO categories; **(B)** molecular function GO categories; **(C)** cellular component GO categories.

(DENV), hepatitis C virus (HCV), rhinoviruses (RhV), rotavirus (RoV), foot-and-mouth disease virus (FMDV), porcine reproductive and respiratory syndrome virus (PRRSV), feline immunodeficiency virus (FIV), canine distemper virus (CDV), and so on in humans and animals (Table 2). Of these virus receptor proteins identified in MPs of Vero cells, the integrin proteins were the common receptors in a variety of viruses.

Discussion

The MPs of the cells become an interested target for exploring and understanding of entry-associated proteins of viruses in animals and humans. In this study, we identified the protein profiles of the MPs of Vero cells using the shotgun proteomics technique, and analyzed potential entry-

associated proteins of viruses according to the current reports. In our study, a total of 627 proteins were successfully identified in the MP samples of Vero cells, and the identified protein numbers were similar to the recent report of plasma proteomics described by Sun *et al.* (2013). Of 627 proteins, the number of putative or uncharacterized proteins was 10 (1.59%). These putative or uncharacterized proteins will enrich the MP proteomics database of Vero cells. In GO annotation of the 627 proteins, the cellular process is a rich biological process, the binding is a rich molecular function, and the membrane is a rich cellular component. These data demonstrated that the identified MPs of Vero cells were mainly membrane-related proteins. In identified 627 proteins, there remained a high proportion of proteins that had no assigned GO annotation, involving in 51.04% (320/627),

TABLE 2. VIRUS INFECTION-ASSOCIATED PROTEINS IN IDENTIFIED MEMBRANE PROTEINS OF VERO CELLS

GO annotation						
No.	Protein name	Protein ID	Biological process	Molecular function	Cellular component	Viruses as receptor
1	Cell adhesion molecule 1 ^a	H1ZZW1	Cell adhesion (GO:0007155)	Receptor binding (GO:0005102)	Plasma membrane (GO:0005886)	RhV (Staunton <i>et al.</i> , 1999), ReV (Barton <i>et al.</i> , 2001), CAV21 (Shafren <i>et al.</i> , 1997), RV (Thoulouze <i>et al.</i> , 1998), HTLV-1 (Hildreth <i>et al.</i> , 1997)
2	Claudin-1/3/4/6/9	A5[S]9/O14493	Cellular process (GO:0009987); multiorganism process (GO:0051704); biological adhesion (GO:0022610)	Binding (GO:0005488)	Membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464)	DENV (Gao <i>et al.</i> , 2010), HCV (Evans <i>et al.</i> , 2007)
3	Integrin $\alpha 3/\alpha 5/\beta 1/\beta 3/\beta 4/\beta 6$	P26006/A5YM53/P05556/P05106/P16144/P18564	Cellular process (GO:0009987); biological adhesion (GO:0022610)	Binding (GO:0005488)	Cell surface (GO:0009986); membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464); protein complex (GO:0043234); cell leading edge (GO:0031252); cell projection (GO:0042995); vesicle (GO:0031982); cell fraction (GO:0000267); membrane-bounded organelle (GO:0043227); intracellular organelle (GO:0043229); intracellular (GO:0005622)	HCMV (Wang <i>et al.</i> , 2005), WSSV (Li <i>et al.</i> , 2007), RoV (Guerrero <i>et al.</i> , 2000), CVB1 (Agrez <i>et al.</i> , 1997), HTNV (Mou <i>et al.</i> , 2006), FMDV (Jackson <i>et al.</i> , 2002), HPV B19 (Weigel-Kelley <i>et al.</i> , 2003), HTNV (Cavri-lovskaya <i>et al.</i> , 1999), HadV (Mathias <i>et al.</i> , 1994), WNV (Chu <i>et al.</i> , 2004), AAV-2 (Summerford <i>et al.</i> , 1999), HSV-1 (Parry <i>et al.</i> , 2005)
4	CD9 ^a	G8JLH6	—	—	Membrane (GO:0016020)	CDV (Löffler <i>et al.</i> , 1997), FIV (Willett <i>et al.</i> , 1994)
5	Epidermal growth factor receptor ^a	E9PFD7	Cell morphogenesis (GO:0009902); signal transduction (GO:0007165)	Nucleotide binding (GO:0000166); protein kinase activity (GO:0004672); ATP binding (GO:0005524)	Membrane (GO:0016020); plasma membrane (GO:0005886)	HCMV (Wang <i>et al.</i> , 2003)
6	Prohibitin	A8K401	Cellular process (GO:0009987); cellular component organization (GO:0016043)	Binding (GO:0005488)	Membrane (GO:0016020); organelle membrane (GO:0031090); organelle envelope (GO:0031967); membrane part (GO:0044425); intracellular (GO:0044446); organelle part (GO:0044422); membrane-bounded organelle (GO:0043227); intracellular organelle (GO:0043229); cell part (GO:0044464); intracellular part (GO:0044424); intracellular (GO:0005622)	CHIKV (Wintachai <i>et al.</i> , 2012), DENV-2 (Kuadkitkan <i>et al.</i> , 2010)

(continued)

TABLE 2. (CONTINUED)

No.	Protein name	Protein ID	GO annotation			Viruses as receptor
			Biological process	Molecular function	Cellular component	
7	Poliovirus receptor ^a	P15151	Cell adhesion (GO:0007155) virus-host interaction (GO:0019048)	Receptor activity (GO:0004872); protein binding (GO:0005515)	Membrane (GO:0016020); extracellular region (GO:0005576)	PRV and BHV-1 (Geraghty <i>et al.</i> , 1998)
8	Neutral amino acid transporter A ^a	P43007	Transport (GO:0006810)	Neutral amino acid transmembrane transporter activity (GO:0015175); sodium: dicarboxylate symporter activity (GO:0017153)	Membrane (GO:0016020); plasma membrane (GO:0005886)	BaERV (Marin <i>et al.</i> , 2000), FERV (Taylor <i>et al.</i> , 1999), HERV (Lavillette <i>et al.</i> , 2002)
9	Syndecan	B4DQ56	—	Binding (GO:0005488)	Membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464)	HIV-1 (Saphire <i>et al.</i> , 2001)
10	Phospholipid scramblase 1/2/3	O15162/ Q7Z4L7/ Q9NRY6	Cellular process (GO:0009987); multiorganism process (GO:0051704); cellular component organization (GO:0016043)	Binding (GO:0005488); transporter activity (GO:0005215)	Membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464)	HCV (Gong <i>et al.</i> , 2011)
11	Coxsackievirus and adenovirus receptor	P78310	Cellular process (GO:0009987); multiorganism process (GO:0051704); cellular component organization (GO:0016043); biological adhesion (GO:0022610)	Binding (GO:0005488)	Membrane (GO:0016020); membrane part (GO:0044425); membrane-bounded organelle (GO:0043227); intracellular organelle (GO:0043229); cell part (GO:0044464); intracellular part (GO:0044424); intracellular (GO:0005622)	CAV and ADV (Cohen <i>et al.</i> , 2001)
12	Carboxypeptidase D	O75976	—	Catalytic activity (GO:0003824); binding (GO:0005488)	Membrane (GO:0016020); membrane part (GO:0044425); intracellular organelle part (GO:0044446); organelle part (GO:0044422); cell fraction (GO:0000267); membrane-bounded organelle (GO:0043227); intracellular organelle (GO:0043229); cell part (GO:0044464); intracellular part (GO:0044424); intracellular (GO:0005622)	AHBV (Tong <i>et al.</i> , 1999)

(continued)

TABLE 2. (CONTINUED)

No.	Protein name	Protein ID	GO annotation			Viruses as receptor
			Biological process	Molecular function	Cellular component	
13	CD151	P48509	Cellular process (GO:0009987); biological adhesion (GO:0022610)	Binding (GO:0005488)	Membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464)	PRRSV (Shanmukhappa <i>et al.</i> , 2007)
14	Niemann-Pick C1 protein	O15118	Bile acid metabolic process (GO:0008206)	Hedgehog receptor activity (GO:0008158)	Membrane (GO:0016020); organelle envelope (GO:0031967); membrane part (GO:0044425); endomembrane system (GO:0012505); intracellular organelle part (GO:0044446); organelle part (GO:0044422); membrane-bounded organelle (GO:0043227); intracellular organelle (GO:0043229); cell part (GO:0044464); intracellular part (GO:0044424); intracellular (GO:0005622)	EboV (Côté <i>et al.</i> , 2011), HCV (Sainz <i>et al.</i> , 2012)
15	Transferrin receptor protein 1	P02786	Establishment of localization (GO:0051234); localization (GO:0051179); cellular process (GO:0009987); multiorganism process (GO:0051704); cellular component organization (GO:0016043)	—	Membrane (GO:0016020); membrane part (GO:0044425); vesicle (GO:0031982); membrane-bounded organelle (GO:0043227); intracellular organelle (GO:0043229); cell part (GO:0044464); intracellular part (GO:0044424); intracellular (GO:0005622)	NWARV (Radoshitzky <i>et al.</i> , 2007), CPV and FPV (Parker <i>et al.</i> , 2001), MMTV (Ross <i>et al.</i> , 2002)
16	Galectin-1	P09382	Cellular process (GO:0009987); death (GO:0016265)	Binding (GO:0005488)	Cell part (GO:0044464); intracellular part (GO:0044424); intracellular (GO:0005622)	HIV-1 (Ouellet <i>et al.</i> , 2005)
17	Junctional adhesion molecule C	Q9BX67	—	—	Membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464)	ReV (Barton <i>et al.</i> , 2001), FCV (Makino <i>et al.</i> , 2006)

^aThese proteins have no GO annotation on the online DAVID Web tool, and their GO annotation was searched on the database of UniProtKB (www.uniprot.org/) according to complete GO annotation (Dimmer *et al.*, 2012).

RV, rabies virus; DENV, dengue virus; HCV, hepatitis C virus; RhV, rhinoviruses; RoV, rotavirus; FMDV, foot-and-mouth disease virus; PRRSV, porcine reproductive and respiratory syndrome virus; FIV, feline immunodeficiency virus; CDV, canine distemper virus.

43.22% (271/627), and 33.97% (213/627) in terms of biological process, molecular function, and cellular components, respectively. This was due to the limitation of the coverage of the current GO annotation system. Although the total number of proteins identified from the MPs of Vero cells was similar to the number identified in human and animal plasma by the shotgun proteomics technique (He *et al.*, 2005; Sun *et al.*, 2013), the identified 627 proteins could not cover all the MPs located on Vero cells due to the complexity and extraction efficiency of MPs, and limitation of the shotgun LC-MS/MS approach.

In 627 identified proteins in MPs of Vero cells, seventeen proteins had been revealed to be virus receptor proteins. Of these virus receptor proteins, the integrin proteins were common receptors in a variety of viruses. Integrins are obligate heterodimers containing two distinct chains, called the α (alpha) and β (beta) subunits (Humphries *et al.*, 2000). Through different combinations of the α and β subunits, some 24 unique integrins are generated, although the number varies according to different studies (Hynes *et al.*, 2002). The current research indicates that $\alpha V\beta 3$ integrin is the cellular receptor of RoV, human cytomegalovirus, west nile virus, and herpes simplex virus type 1 (Guerrero *et al.*, 2000; Chu and Ng, 2004; Parry *et al.*, 2005; Wang *et al.*, 2005); β integrin is the cellular receptor of white spot syndrome virus (Li *et al.*, 2007); $\alpha V\beta 6$ integrin is the cellular receptor of coxsackievirus B1 (Agrez *et al.*, 1997); $\beta 3$ integrin is the cellular receptor of hantaan virus (Mou *et al.*, 2006); $\alpha V\beta 1$ and $\alpha V\beta 6$ integrins are the cellular receptors of foot-and-mouth disease virus (Jackson *et al.*, 2002); $\alpha V\beta 1$ integrin is the cellular receptor of human parvovirus B19 (Weigel-Kelley *et al.*, 2003); $\beta 3$ integrin is the cellular receptor of hantaviruses (Gavrilovskaya *et al.*, 1999); αV integrin is the cellular receptor of human adenovirus (Mathias *et al.*, 1994); $\alpha V\beta 5$ integrin is the cellular receptor of adeno-associated virus (Summerford *et al.*, 1999). These data revealed that different combinations of α subunits and β subunits in integrin proteins were used as the receptors of the different viruses. It is supposed that the integrin protein may be involved in a wide range of virus-entry activities through variants of some of the subunits formed by differential splicing (Stewart *et al.*, 2007). The integrin protein here was highlighted as an interested target for exploration of the entry factors for understanding of virus infection of Vero cells. Additionally, the remaining 16 proteins should be also concerned as the target of virus receptors, especially cell adhesion molecule 1, transferrin receptor protein 1, and neutral amino acid transporter A, claudin, CD9, prohibitin, Niemann-Pick C1 protein, and junctional adhesion molecule C.

In the current study, we only provide some basic information of virus entry-related proteins in Vero cells using the shotgun LC-MS/MS proteomic tool, and make a preliminary analysis or speculation regarding potential proteins responsible for the virus entry. However, the role of seventeen virus receptor-related proteins in the virus entry, especially the highlighted integrin protein, still need to be verified in future research.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (Grant No. 31001081/31310103022), the

Program for New Century Excellent Talents in the Heilongjiang Provincial University (Grant No. 1252-NCET-016), and the Technological Innovation Team Building Program of University of Heilongjiang Province (Grant No. 2010td05). We also thank the Research Center for Proteome Analysis of Shanghai Institutes for Biological Sciences, Shanghai, China, for help in protein analysis.

Disclosure Statement

The authors have no financial interests to disclose.

References

- Agrez, M.V., Shafren, D.R., Gu, X., Cox, K., Sheppard, D., and Barry, R.D. (1997). Integrin $\alpha v \beta 6$ enhances coxsackievirus B1 lytic infection of human colon cancer cells. *Virology* **239**, 71–77.
- Barton, E.S., Forrest, J.C., Connolly, J.L., Chappell, J.D., Liu, Y., Schnell, F.J., Nusrat, A., Parkos, C.A., and Dermody, T.S. (2001). Junction adhesion molecule is a receptor for reovirus. *Cell* **104**, 441–451.
- Cheng, B.Y., Ortiz-Riaño, E., de la Torre, J.C., and Martínez-Sobrido, L. (2013). Generation of recombinant arenavirus for vaccine development in FDA-approved Vero cells. *J Vis Exp*. 2013. [Epub ahead of print]; DOI: 10.3791/50662.
- Chu, J.J., and Ng, M.L. (2004). Interaction of West Nile virus with $\alpha v \beta 3$ integrin mediates virus entry into cells. *J Biol Chem* **279**, 54533–54541.
- Cohen, C.J., Shieh, J.T., Pickles, R.J., Okegawa, T., Hsieh, J.T., and Bergelson, J.M. (2001). The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proc Natl Acad Sci USA* **98**, 15191–15196.
- Côté, M., Misasi, J., Ren, T., Bruchez, A., Lee, K., Filone, C.M., Hensley, L., Li, Q., Ory, D., Chandran, K., and Cunningham, J. (2011). Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. *Nature* **477**, 344–348.
- Dimmer, E.C., Huntley, R.P., Alam-Faruque, Y., Sawford, T., O'Donovan, C., and Martin, M.J. (2012). The UniProt-GO Annotation database in 2011. *Nucleic Acids Res* **40**, D565–D570.
- Evans, M.J., von Hahn, T., Tscherne, D.M., Syder, A.J., Panis, M., Wölk, B., Hatzioannou, T., McKeating, J.A., Bieniasz, P.D., and Rice, C.M. (2007). Claudin-1 is a hepatitis C virus coreceptor required for a late step in entry. *Nature* **446**, 801–805.
- Gao, F., Duan, X., Lu, X., Liu, Y., Zheng, L., Ding, Z., and Li, J. (2010). Novel binding between pre-membrane protein and claudin-1 is required for efficient dengue virus entry. *Biochem Biophys Res Commun* **391**, 952–957.
- Gavrilovskaya, I.N., Brown, E.J., Ginsberg, M.H., and Mackow, E.R. (1999). Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by $\beta 3$ integrins. *J Virol* **73**, 3951–3959.
- Geraghty, R.J., Krummenacher, C., Cohen, G.H., Eisenberg, R.J., and Spear, P.G. (1998). Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. *Science* **280**, 1618–1620.
- Gong, Q., Cheng, M., Chen, H., Liu, X., Si, Y., Yang, Y., Yuan, Y., Jin, C., Yang, W., He, F., and Wang, J. (2011). Phospholipid scramblase 1 mediates hepatitis C virus entry into host cells. *FEBS Lett* **585**, 2647–2652.
- Guerrero, C.A., Méndez, E., Zárate, S., Isa, P., López, S., and Arias, C.F. (2000). Integrin $\alpha(v)\beta(3)$ mediates rotavirus cell entry. *Proc Natl Acad Sci USA* **97**, 14644–14649.
- He, P., He, H.Z., Dai, J., Wang, Y., Sheng, Q.H., Zhou, L.P., Zhang, Z.S., Sun, Y.L., Liu, F., Wang, K., Zhang, J.S., Wang,

- H.X., Song, Z.M., Zhang, H.R., Zeng, R., and Zhao, X. (2005). The human plasma proteome: analysis of Chinese serum using shotgun strategy. *Proteomics* **5**, 3442–3453.
- Hildreth, J.E., Subramaniam, A., and Hampton, R.A. (1997). Human T-cell lymphotropic virus type 1 (HTLV-1)-induced syncytium formation mediated by vascular cell adhesion molecule-1: evidence for involvement of cell adhesion molecules in HTLV-1 biology. *J Virol* **71**, 1173–1180.
- Huang, D.W., Sherman, B.T., and Lempicki, R.A. (2009a). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* **37**, 1–13.
- Huang, D.W., Sherman, B.T., and Lempicki, R.A. (2009b). Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc* **4**, 44–57.
- Humphries, M.J. (2000). Integrin structure. *Biochem Soc Trans* **28**, 311–339.
- Hynes, R. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673–687.
- Jackson, T., Mould, A.P., Sheppard, D., and King, A.M. (2002). Integrin alphavbeta1 is a receptor for foot-and-mouth disease virus. *J Virol* **76**, 935–941.
- Jolly, C.L., and Sattentau, Q.J. (2013). Attachment factors. *Adv Exp Med Biol* **790**, 1–23.
- Kuadkitkan, A., Wikan, N., Fongsaran, C., and Smith, D.R. (2010). Identification and characterization of prohibitin as a receptor protein mediating DENV-2 entry into insect cells. *Virology* **406**, 149–161.
- Lavillette, D., Marin, M., Ruggieri, A., Mallet, F., Cosset, F.L., and Kabat, D. (2002). The envelope glycoprotein of human endogenous retrovirus type W uses a divergent family of amino acid transporters/cell surface receptors. *J Virol* **76**, 6442–6452.
- Li, D.F., Zhang, M.C., Yang, H.J., Zhu, Y.B., and Xu, X. (2007). Beta-integrin mediates WSSV infection. *Virology* **368**, 122–132.
- Löffler, S., Lottspeich, F., Lanza, F., Azorsa, D.O., ter Meulen, V., and Schneider-Schaulies, J. (1997). CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. *J Virol* **71**, 42–49.
- Makino, A., Shimajima, M., Miyazawa, T., Kato, K., Tohya, Y., and Akashi, H. (2006). Junctional adhesion molecule 1 is a functional receptor for feline calicivirus. *J Virol* **80**, 4482–4490.
- Marin, M., Taylor, C.S., Nouri, A., and Kabat, D. (2000). Sodium-dependent neutral amino acid transporter type 1 is an auxiliary receptor for baboon endogenous retrovirus. *J Virol* **74**, 8085–8093.
- Mathias, P., Wickham, T., Moore, M., and Nemerow, G. (1994). Multiple adenovirus serotypes use alpha v integrins for infection. *J Virol* **68**, 6811–6814.
- Mou, D.L., Wang, Y.P., Huang, C.X., Li, G.Y., Pan, L., Yang, W.S., and Bai, X.F. (2006). Cellular entry of Hantaan virus A9 strain: specific interactions with beta3 integrins and a novel 70 kDa protein. *Biochem Biophys Res Commun* **339**, 611–617.
- Ouellet, M., Mercier, S., Pelletier, I., Bounou, S., Roy, J., Hirabayashi, J., Sato, S., and Tremblay, M.J. (2005). Galectin-1 acts as a soluble host factor that promotes HIV-1 infectivity through stabilization of virus attachment to host cells. *J Immunol* **174**, 4120–4126.
- Ozaki, H., Govorkova, E.A., Li, C., Xiong, X., Webster, R.G., and Webby, R.J. (2004). Generation of high-yielding influenza A viruses in African green monkey kidney (Vero) cells by reverse genetics. *J Virol* **78**, 1851–1857.
- Pan, Y., Tian, X., Li, W., Zhou, Q., Wang, D., Bi, Y., Chen, F., and Song, Y. (2012). Isolation and characterization of a variant porcine epidemic diarrhea virus in China. *Virol J* **9**, 195.
- Parker, J.S., Murphy, W.J., Wang, D., O'Brien, S.J., and Parrish, C.R. (2001). Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter, and infect cells. *J Virol* **75**, 3896–3902.
- Parry, C., Bell, S., Minson, T., and Browne, H. (2005). Herpes simplex virus type 1 glycoprotein H binds to alphavbeta3 integrins. *J Gen Virol* **86**, 7–10.
- Radoshitzky, S.R., Abraham, J., Spiropoulou, C.F., Kuhn, J.H., Nguyen, D., Li, W., Nagel, J., Schmidt, P.J., Nunberg, J.H., Andrews, N.C., Farzan, M., and Choe, H. (2007). Transferrin receptor 1 is a cellular receptor for New World haemorrhagic fever arenaviruses. *Nature* **446**, 92–96.
- Ross, S.R., Schofield, J.J., Farr, C.J., and Bucan, M. (2002). Mouse transferrin receptor 1 is the cell entry receptor for mouse mammary tumor virus. *Proc Natl Acad Sci USA* **99**, 12386–12390.
- Sainz, B., Jr., Barretto, N., Martin, D.N., Hiraga, N., Imamura, M., Hussain, S., Marsh, K.A., Yu, X., Chayama, K., Alrefai, W.A., and Uprichard, S.L. (2012). Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. *Nat Med* **18**, 281–285.
- Saphire, A.C., Bobardt, M.D., Zhang, Z., David, G., and Galloway, P.A. (2001). Syndecans serve as attachment receptors for human immunodeficiency virus type 1 on macrophages. *J Virol* **75**, 9187–9200.
- Shafren, D.R., Dorahy, D.J., Ingham, R.A., Burns, G.F., and Barry, R.D. (1997). Coxsackievirus A21 binds to decay-accelerating factor but requires intercellular adhesion molecule 1 for cell entry. *J Virol* **71**, 4736–4743.
- Shanmukhappa, K., Kim, J.K., and Kapil, S. (2007). Role of CD151, A tetraspanin, in porcine reproductive and respiratory syndrome virus infection. *Virol J* **4**, 62.
- Staunton, D.E., Merluzzi, V.J., Rothlein, R., Barton, R., Marlin, S.D., and Springer, T.A. (1989). A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* **56**, 849–853.
- Stewart, P.L., and Nemerow, G.R. (2007). Cell integrins: commonly used receptors for diverse viral pathogens. *Trends Microbiol* **15**, 500–507.
- Summerford, C., Bartlett, J.S., and Samulski, R.J. (1999). AlphaVbeta5 integrin: a co-receptor for adeno-associated virus type 2 infection. *Nat Med* **5**, 78–82.
- Sun, D., Zhang, H., Guo, D., Sun, A., and Wang, H. (2013). Shotgun proteomic analysis of plasma from dairy cattle suffering from footrot: characterization of potential disease-associated factors. *PLoS One* **8**, e55973.
- Taylor, C.S., Nouri, A., Zhao, Y., Takeuchi, Y., and Kabat, D. (1999). A sodium-dependent neutral-amino-acid transporter mediates infections of feline and baboon endogenous retroviruses and simian type D retroviruses. *J Virol* **73**, 4470–4474.
- Thoulouze, M.I., Lafage, M., Schachner, M., Hartmann, U., Cremer, H., and Lafon, M. (1998). The neural cell adhesion molecule is a receptor for rabies virus. *J Virol* **72**, 7181–7190.
- Tong, S., Li, J., and Wands, J.R. (1999). Carboxypeptidase D is an avian hepatitis B virus receptor. *J Virol* **73**, 8696–8702.
- Vlecken, D.H., Pelgrim, R.P., Ruminski, S., Bakker, W.A., and van der Pol, L.A. (2013). Comparison of initial feasibility of host cell lines for viral vaccine production. *J Virol Methods* **193**, 28–41.
- Wang, X., Huang, D.Y., Huong, S.M., and Huang, E.S. (2005). Integrin alphavbeta3 is a coreceptor for human cytomegalovirus. *Nat Med* **11**, 515–521.
- Wang, X., Huong, S.M., Chiu, M.L., Raab-Traub, N., and Huang, E.S. (2003). Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. *Nature* **424**, 456–461.

- Weigel-Kelley, K.A., Yoder, M.C., and Srivastava, A. (2003). Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of beta1 integrin for viral entry. *Blood* **102**, 3927–3933.
- Willett, B.J., Hosie, M.J., Jarrett, O., and Neil, J.C. (1994). Identification of a putative cellular receptor for feline immunodeficiency virus as the feline homologue of CD9. *Immunology* **81**, 228–233.
- Wintachai, P., Wikan, N., Kuadkitkan, A., Jaimipuk, T., Ubol, S., Pulmanasahakul, R., Auewarakul, P., Kasinrerak, W., Weng, W.Y., Panyasrivanit, M., Paemanee, A., Kittisenachai, S., Roytrakul, S., and Smith, D.R. (2012). Identification of prohibitin as a Chikungunya virus receptor protein. *J Med Virol* **84**, 1757–1770.
- Wiśniewski, J.R., Zougman, A., Nagaraj, N., and Mann, M. (2009). Universal sample preparation method for proteome analysis. *Nat Methods* **6**, 359–362.
- Yasumura, Y., and Kawakita, M. (1963). The research for the SV40 by means of tissue culture technique. *Nippon Rinsho* **21**, 1201–1219.
- Yim, E.K., Meoyng, J., Namakoong, S.E., Um, S.J., and Park, J.S. (2004). Genomic and proteomic expression patterns in HPV-16 E6 gene transfected stable human carcinoma cell lines. *DNA Cell Biol* **23**, 826–835.
- Zhou, F., Zhou, J., Ma, L., Song, S., Zhang, X., Li, W., Jiang, S., Wang, Y., and Liao, G. (2012). High-yield production of a stable Vero cell-based vaccine candidate against the highly pathogenic avian influenza virus H5N1. *Biochem Biophys Res Commun* **421**, 850–854.

Address correspondence to:

Dongbo Sun, PhD

College of Animal Science and Veterinary Medicine

Heilongjiang Bayi Agricultural University

Daqing High-Tech Industrial Development Zone

Daqing 163319

People's Republic of China

E-mail: dongbosun@126.com

Received for publication August 8, 2013; received in revised form October 25, 2013; accepted October 25, 2013.