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Research Overview

Infectomics Screening for Novel Antiviral Drug Targets

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ABSTRACT Infectomics, a novel way to globally and comprehensively understand the interactions between microbial pathogens and their hosts, has significantly expanded understanding of the microbial infections. The infectomics view of viral–host interactions on the viral perspective principally focuses on gene acquisition, deletion, and point mutation, while traditional antiviral drug discovery concentrates on viral encoding proteins. Recently, high-throughput technologies, such as mass spectrometry-based proteomics, activity-based protein profiling, microarray analysis, yeast two-hybrid assay, small interfering RNA screening, and micro RNA profiling, have been gradually employed in the research of virus–host interactions. Besides, signaling pathways and cellular processes involved in viral–host interactions provide new insights of infectomics in antiviral drug discovery. In this review, we summarize related infectomics approaches in the studies of virus–host interactions, which shed light on the development of novel antiviral drug targets screening. Drug Dev Res 73 : 365–380, 2012.

Key words: infectomics; drug targets; viral–host interactions

INTRODUCTION

Viruses, as small infectious agent, infect nearly all organisms and can cause severe infectious diseases in human. The use of chemical therapy (e.g., ribavirin) or vaccines has proved effective in protecting against viral infections, but some infections like human immunodeficiency virus (HIV) [Cohen et al., 2011], hepatitis B virus (HBV) [Dienstag, 2008], and influenza viruses [Lambert and Fauci, 2010] remain a great challenge. With progress in Genome Sequencing Project, the whole-genome complete genomes of 2,837 viruses have been sequenced at the time of writing this review (http://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=10239&opt=Virus), leading to a new era of viral genomics and proteomics [Burley et al., 1999; Pandey and Mann, 2000; Dongre et al., 2001; Collins et al., 2003; Tyers and Mann, 2003]. Previous studies, mainly based on genomic and proteomic approaches, have made significant progress in establishing the foundation of network-based investigations on viral–host interactions [Chakravarti et al., 2000; Wilson and Richardson, 2005; Wu et al., 2005].

Infectomics, a term first introduced by Huang et al., 2002], is a novel means to globally and comprehensively understand interactions between microbial pathogens and their hosts rather than microbial pathogens themselves. Three types of infectomics approaches have been developed for antimicrobial drug discovery: ecological infectomics, immunoinfectomics, and chemical infectomics [Huang et al., 2007]. Among them, the accelerated development of chemical-based infectomics approaches has greatly contributed to drug discovery efforts. High-throughput approaches that

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include mass spectrometry (MS)-based proteomics, activity-based protein profiling (ABPP), microarray analysis, yeast two-hybrid (YTH) assays, small interfering RNA (siRNA) screening, and micro RNA (miRNA) profiling, are used to study the infectomes related to viral infection, leading to detailed mechanisms of viral–host interactions, including HIV [Brass et al., 2008; König et al., 2008; Naji et al., 2012], hepatitis C virus (HCV) [Supekova et al., 2008; Peng et al., 2009], influenza virus [König et al., 2009], and dengue virus (DENV) [Khadka et al., 2011].

Recent progress in antiviral drug research based on infectomics approaches has greatly revolutionized existing strategies. New antiviral compounds targeting host gene-encoding proteins or enzymes are in preclinical research, e.g., PRO2000 [McCormack et al., 2010] and bisindolylmaleimide [Ludwig et al., 2003]. As nuclear receptors play a combinatorial role in inflammation and immunity [Glass and Ogawa, 2005], novel drugs targeting these represent a potential strategy for antiviral therapy. Targeting infectome cellular proteins or molecules may lead to reduced host drug resistance, as the human genes encoding targeted cellular proteins are less likely to mutate in response to therapy [Tan et al., 2007]. Infectomics approaches can be used to study complex viral–host interactions to facilitate screening novel antiviral drug targets. In this review, we focus on high-throughput infectomic approaches in the study of virus–host interactions and their potential in antiviral drug discovery.

THE VIRAL PERSPECTIVE IN DRUG DISCOVERY

In the past few decades, many publications involving viral infections have focused on genomic and proteomic approaches from a viral perspective. The gene acquisition, deletion, and point mutation are three major events leading to the evolution of microbial pathogens or commensals. Point mutation is the most frequent leading to the promotion of viral replication and drug resistance. The survival ability of some viruses is enhanced due to viral point mutation, indicating that point mutations may play a critical role in virus spread. For example, in spite of the presence of antibodies against e antigen in the serum, patients infected with HBV still have a high titer of HBV DNA. Akahane et al. [1990] by sequencing the precore region of HBV, identified a point mutation at nucleotide 83 that was present in 98% clones of HBV propagated from the sera of seven patients, and another mutation at nucleotide 86 in 29 clones from two patients, which were responsible for dysfunctional secretion of e antigen. This point mutation is not limited to HBV, but is a general viral phenomenon that has been identified in a variety of viruses, including HIV [Emiliani et al., 1996], HCV [Heilek and Peterson, 1997], DENV [Hanley et al., 2003], influenza virus [Melikyan et al., 2000], and herpes simplex virus (HSV) [Hwang et al., 1992].

Using genomic and proteomic approaches, significant progress in drug discovery based on viral encoding proteins has occurred. Gel electrophoresis and liquid chromatography-MS/MS technologies resulted in the discovery of many proteins that may represent potential drug targets and include viral enzymes like reverse transcriptase (RT), integrase, and protease, which are involved in virus binding, reverse transcription, integration, and budding. For instance, the use of HIV-1 protease inhibitors, like saquinavir, ritonavir, indinavir, and nelfinavir have improved the treatment of HIV-infected patients [Deeks et al., 1997; Eron, 2000]. However, many patients treated with HIV-1 protease inhibitors developed tolerance [Condra et al., 1995; Yerly et al., 1999] requiring an enhanced knowledge of the landscape of viral infections. Furthermore, host factors that play essential roles in viral infections could also serve as novel antiviral drugs.

THE HOST PERSPECTIVE IN DRUG DISCOVERY

To date, virus–host interactions require additional characterization, as previous studies of viral infections focused mainly on the viruses themselves leading to antiviral drugs that targeted viral proteins with inherent limitations, e.g., rapid resistance due to the viral type and low fidelity of viral replication, especially for the RNA viruses [Drake et al., 1998]) [Friedel and Haas, 2011]. Furthermore, viral genomes represent a limited number of drug targets [Tisoncik et al., 2009]. With novel high-throughput technologies, host factors essential for viral infections have been identified. A summary of the currently used high-throughput technologies and their contributions to antiviral drug discovery follows.

MS-Based Proteomics

Proteome analysis (primary sequence, protein–protein interactions, posttranslational modifications, etc.) can be used to study cellular states and determine molecular aspects of cellular function. Owing to the complexity of proteins and their low abundance, MS-based proteomics [Rabilloud, 2002; Monteoliva and Albar, 2004; Righetti et al., 2004] is an indispensable tool in viral systems biology (Fig. 1A) [Aebersold and Mann, 2003]. Ion traps [Fenn et al., 1989; Pitteri et al., 2005; Makarov et al., 2006; Second et al., 2009; Shaner et al., 2009], time-of-flight (TOF) [Marklein et al., 2009; Seng et al., 2009; Prod'hom et al., 2010],

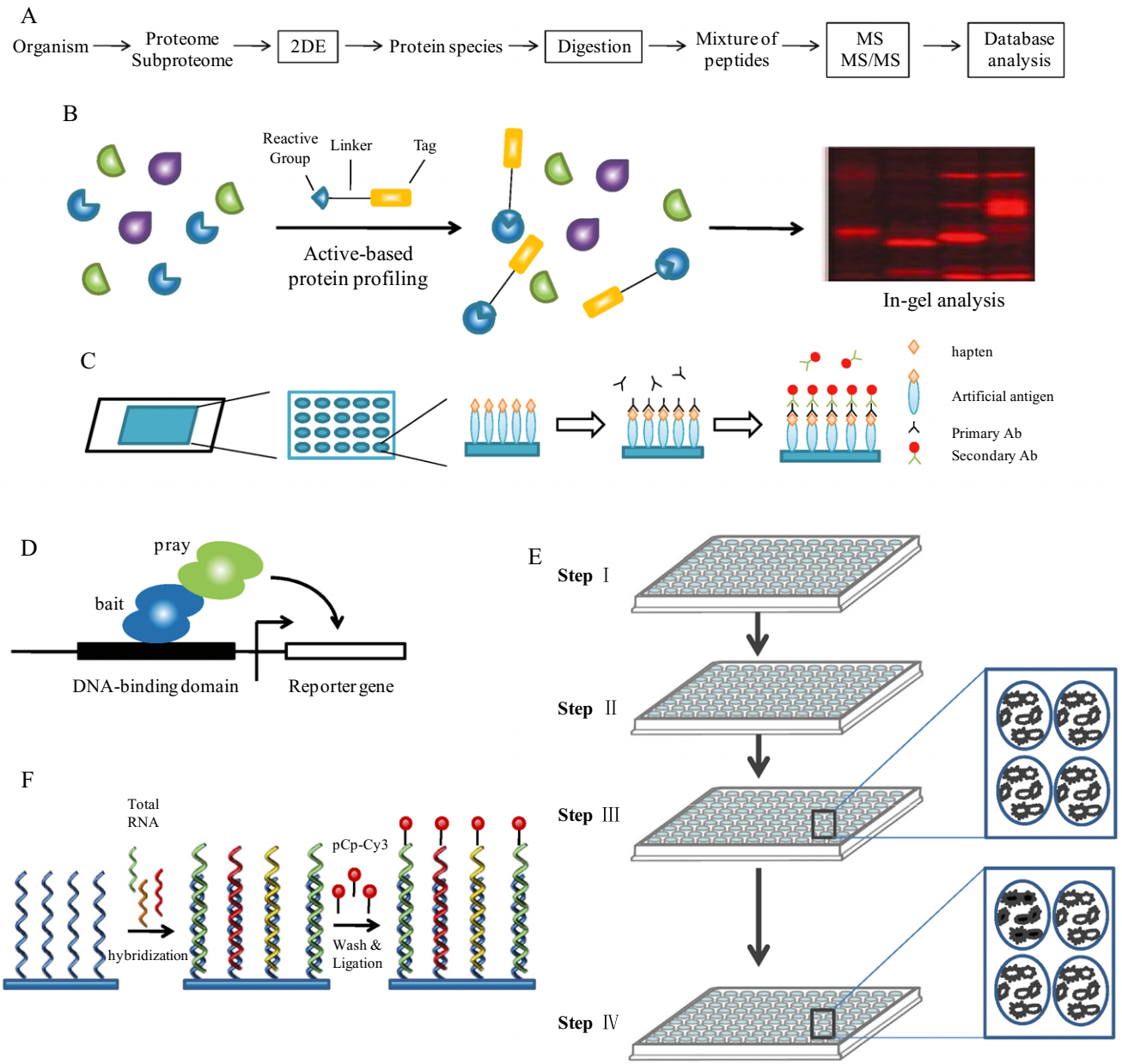

Fig. 1. High-throughput technologies and omics approaches currently used in novel drug target screening. Six technologies are commonly used in drug discovery. (**A**) MS-based proteomic approach. (**B**) Schematic view of activity-based protein profiling (ABPP). Cell lysates are mixed with activity-directed chemical probes targeting candidate proteins, and then the tags of the probes could be detected by in-gel analysis. (**C**) Schematic view of protein microarray analysis. First, the artificial antigens are attached on the solid substrate. Then, the primary antibodies are deposited to the hapten. Finally, the primary antibodies bind to the secondary antibodies with the labels for detection. (**D**) Schematic view of yeast two-hybrid assay (YTH). The protein of interest (bait) is fused to a DNA-binding domain and transfected in a yeast host cell that contains a reporter gene controlled by this DNA-binding domain. The functional transcription factor (TF) will reconstitute upon the physical interaction between bait and prey proteins, leading to the activation of the reporter gene. (**E**) Schematic view of siRNA screening. Step I, preparation of siRNAs; step II, rearray siRNAs into 384-well plates for high-throughput screening; step III, transfection of siRNAs into target cell lines; and step IV assay phenotype by identifying the plate position (see black cell). (**F**) Schematic view of miRNA profiling. The total RNAs are hybridized with the miRNAs. Then the RNAs are labeled with the pCp-Cy3 for detection. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

quadruple [Abzalimov and Kaltashov, 2010; Ramanathan et al., 2011], and Fourier transform-MS ion cyclotron analyzers [Leon et al., 2009; Allwood et al., 2012] are thee four basic types of mass analyzers used in proteomic investigation. There are multiple options available to collect data and analysis including isotope-coded affinity tag [Haqqani et al., 2005], stable isotope labeling with amino acids in cell culture [Asara

et al., 2008], and N15 [Osserman et al., 1957]. As more viral and host genomes are sequenced, MS-based proteomics is employed in research on virus–host interactions. Two-dimensional electrophoresis (2DE)/MS-based proteomics are powerful tools in infectomics research. Matrix-assisted laser desorption/ionization-TOF-MS has been successfully used in combination with 2DE for infectomics analyses of the *Chlamydia pneumonia* elementary body in Hep-2 cells and *C. trachomatis* reticulate body in HeLa 229 cells [Huang et al., 2002]. Of the HCV nonstructural proteins, nonstructural protein 5A (NS5A) plays a critical role in RNA binding [Huang et al., 2005] and replication [Tarao et al., 2005; Targett-Adams et al., 2008], but its exact role remains unknown, particularly in virus–host interactions. Choi et al., 2004] identified heat shock protein 27 (HSP27) as a protein that specifically co-immnoprecipitated with NS5A but not with NS5B using MS analysis and other approaches. Moreover, the N-terminal regions of NS5A (aa 1–181) was found to interact with the 1–122 amino acid domain of HSP27. When heat shocked, HSP27 and NS5A co-localize to the endoplasmic reticulum suggesting an important role during heat shock. Kou et al., 2006] examined the possibility of host factors, that inhibit translation in cultured cells, to interact with NS4A [Libbus et al., 2002; Stasko et al., 2002]. Glutathione S-transferase-NS4A interacting protein was found to be congruent with human translation eukaryotic elongation factor 1A (eEF1A). Furthermore, the central domain from residues 21–34 of NS4A interacted with eEF1A, causing inhibition of both cap-dependent and HCV internal ribosomal entry site-mediated translation activities.

In HIV-1 infectious disease, MS-based proteomic research has also made significant progress. HIV-1 trans-activating (Tat) protein is necessary for viral replication and may play a critical part in HIV-1-associated diseases [Rasheed et al., 2009]. Interestingly, after HIV-1 infection, T cells were protected against apoptosis and survive longer bearing virions. Although the precise mechanism is unclear, it appears likely that HIV-1 Tat protein is responsible for such protection [Coiras et al., 2006]. Using MS–based proteomics, a number of cytoskeletal proteins, e.g., β-tubulin, actin, gelsolin, cofilin, annexin II, and Rac/Rho-GDI (Rac and Rho-guanine nucleotide dissociation inhibitor) complex, were found to be downregulated by Tat protein. Reduced expression of these proteins limited the cytoskeletal changes induced by apoptosis and thus maintained HIV-1 virions. MS-based approaches have been used to screen potential inhibitors of Tat protein as novel therapeutic agents. Using nuclear magnetic resonance and MS/MS studies, [Jayasuriya et al., 2002 discovered durhamycin A as an inhibitor of Tat transactivation. MS-based proteomics have explored other common viruses, including dengue [Pattanakitsakul et al., 2007; Higa et al., 2008], influenza [Williams et al., 2008; Schwahn et al., 2010], severe acute respiratory syndrome-associated coronavirus [Zeng et al., 2004; Jiang et al., 2005], and human respiratory syncytial virus [Brasier et al., 2004; Munday et al., 2010].

ABPP

ABPP technologies can monitor proteins or enzymes in their native environment, thus eliminating the need for recombinant expression, purification, and the development of a specific assay. Activity-directed chemical probes, which target many members of a given enzyme class or protein family, can be used to evaluate the activity of candidate compounds directly in complex proteomes. Identifying protein function and validating its biologic role is a prerequisite to lead discovery (Fig. 1B). The discovery of reversible enzyme inhibitors can be simplified with this chemical proteomic approach. In order to characterize the specific cellular functions of the cysteine proteases required for survival of the malaria parasite *Plasmodium falciparum*, a chemical proteomic screen was used to characterize these predominant proteases. Falcipain 1 was identified as the only active protease during the invasive merozoite stage. Specific inhibitors for falcipain 1 were then identified by the screening and were able to block parasite invasion of host erythrocytes, suggesting that falcipain 1 played a specific role in host cell invasion with the inhibitor for this enzyme representing a potential new agent for antimalarial therapeutics [Greenbaum et al., 2002].

Microarray Analysis

Microarray analysis, a 2D array on a solid substrate using high-throughput screening methods to assay large amounts of biological material [Barbulovic-Nad et al., 2006], was first used to study the small mustard plant *Arabidopsis thaliana* [Schena et al., 1995] and was then used to study yeast [Shalon et al., 1996], human [Schena et al., 1996], and mouse [Lockhart et al., 1996]. Compared with traditional approaches, the principal advantage of microarrays is that a large number of targets can be analyzed in parallel measurements with low sample consumption (Fig. 1C). Similar to recombinant DNA [Jackson et al., 1972] and polymerase chain reaction (PCR) [Mullis and Faloona, 1987], microarray is a seminal technology with broad application [Stears et al., 2003], including genomics [DeRisi et al., 1997; Hughes et al., 2000; Sudarsanam et al., 2000] and proteomics [Geiss et al., 2000; MacBeath and Schreiber, 2000; Eickhoff et al., 2002]. The two commonly used microarrays are those for DNA and protein [Templin et al., 2002]

where enzyme-substrate [Bulyk et al., 1999; Arenkov et al., 2000; MacBeath and Schreiber, 2000; Zhu et al., 2000], DNA-protein [Bulyk et al., 1999], protein-ligand, and different types of protein–protein interactions [Ge, 2000] are studied. DNA microarrays shifted direct antiviral screening programs to rational and genome-wide target-based strategies [Schmid, 2001; Chan et al., 2002; Fritz and Raczniak, 2002; McDevitt et al., 2002; Parkinson, 2002; Cheng et al., 2003]. Since the first report of microarray-based investigations of HIV-induced alterations in host gene expression [Geiss et al., 2000], microarrays have been used in HIV studies [Izmailova et al., 2003; Khodakov et al., 2008] to investigate macrophage responses of infection by African swine fever virus [Zhang et al., 2006]. Genomic comparison of tuberculosis vaccine strain variants (Bacillus Calmette-Guérin), *Mycobacterium tuberculosis* H37Rv, *Helicobacter pylori*, and methicillin-resistant *Staphylococcus aureus* has been conducted using DNA microarray analysis providing new information on the evolution of these human pathogens suggesting rational approaches to the design of improved diagnostics and antimicrobial agents. However, bridging the gap between genomes and therapeutics is a challenging and time-consuming research process that is rate-limiting [Falb and Jindal, 2002; Zanders et al., 2002]. Protein microarrays also provide a high-throughput platform for target identification. A cluster of secreted proteins (α-defensins 1, 2, and 3) were identified as cell anti-HIV factors (CAFs) [Zhang et al., 2002] that were secreted by cluster of differentiation 8 T-lymphocytes from certain immunologically stable HIV-1 patients to suppress HIV-1 replication. The specific antibody recognition and amino acid sequencing were used to confirm the identity of CAF. Protein microarrays using malaria parasite surface proteins have been developed for studies of parasitic diseases [Bacarese-Hamilton et al., 2002]. The receptor-binding characteristics of two isolates of the novel pandemic H1N1 virus, Cal/09, and A/Hamburg/5/2009 (Ham/09), which were compared directly by carbohydrate microarray analysis [Childs et al., 2009].

YTH Assay

The YTH assay [Fields and Song, 1989] is a high-throughput technology to study protein–protein interactions [Fields, 2005]. In YTH, a protein of interest is fused to a DNA-binding domain and transfected in a yeast host cell with the reporter gene controlled by this DNA-binding domain. This fusion protein can be used as a "bait" or "target" to screen a library of cDNA clones fused to an activation domain. The functional transcription factor will reconstitute upon the physical interaction between bait and prey proteins, activating a reporter gene (Fig. 1D). YTH has been widely used in studying virus–host protein–protein interactions, including *Escherichia coli* bacteriophage 7 [Bartel et al., 1996], HIV [Rossi et al., 1996], and HCV [Matsumoto et al., 1997; Mamiya and Worman, 1999; Kittlesen et al., 2000]. Khadka et al. [2011] used a YTH assay to study network interactions between DENV and human proteins and validated a subset of these interactions through split-luciferase, siRNA, and colocalization experiments, resulting in the first genome-wide analysis of DENV–human protein–protein interactions. They identified 93 proteins required for DENV replication, 60 of which that were not been linked to any other viruses, and showed that some of proteins involved in DENV infection also linked to other viruses, particularly HCV [Khadka et al., 2011]. This study has provided new light on DENV–host interactions as well as new potential drugs targeting host proteins. Mouse hepatitis virus (MHV)-68, a useful model for the study of human γ-herpes viruses, has been studied using YTH with 23 intraviral protein interactions and 243 virus-cellular protein interactions being identified, most of which have never been reported before [Lee et al., 2011]. Such studies may reveal potential cellular proteins that are utilized by MHV-68 or DENV, which may serve as new targets for therapeutic intervention. Studies on stomatitis virus [Moerdyk-Schauwecker et al., 2011], Sesbania mosaic virus [Chowdhury and Savithri, 2011], flavivirus [Le Breton et al., 2011], influenza virus [Sharma et al., 2011; Tafforeau et al., 2011], human cytomegalovirus [To et al., 2011], and human T-lymphotropic virus (HTLV) types 1 and 2 retroviruses [Simonis et al., 2012] also provided novel insights into virus–host interactions.

Variants of YTH include membrane YTH system, split-tobacco etch virus system, and mammalian protein–protein interaction trap [Suter et al., 2008]. Compared with YTH, new technologies like reverse YTH and the yeast three-hybrid system, can provide more integrative data to understand of viral–host interactions and provide new strategies for antiviral drug discovery. For instance, inhibitors of dimerization can be used to disrupt protein–protein interactions induced by viral infections, which may prevent viral infection or replication. Protease, RT, invertase of HIV, and DNA polymerase of HSV, which play critical roles in HIV and HSV infections, are drug targets for HIV and HSV therapies [Archakov et al., 2003].

siRNA Screening

RNA interference (RNAi) is a mechanism within living cells to modulate gene activity [Fire et al., 1998] It is widely used to study gene function and associated

molecular mechanisms [Hannon, 2002; Mello and Conte, 2004]. Since initial studies on large-scale RNAi screening in *Caenorhabditis elegans* [Fraser et al., 2000; Gönczy et al., 2000], RNAi screening has been routinely used to study of pathogen–host interactions, enabling genome-scale loss of function screening in host cells (Fig. 1E) [Echeverri and Perrimon, 2006; Boutros and Ahringer, 2008; Mohr et al., 2010; Ou et al., 2012]. Genome-wide RNAi screening identified 287 human host cell genes influencing influenza A virus replication [Karlas et al., 2010] with host protein p27, a cell cycle regulator, being identified as key for influenza virus replication. A small molecule inhibitor of cell division cycle-like kinase 1 reduced influenza virus replication. Another study identified 295 cellular cofactors required for early-stage influenza virus replication with 23 factors necessary for viral entry confirmed [König et al., 2010]. Some 250 host cellular factors influencing HIV-1 infection have been identified 40 factors participating in the early stage of HIV infection [Brass et al., 2008; König et al., 2008]. High-throughput RNAi screening has identified host factors involved in virus progression, including those for HCV [Li et al., 2009; Tai et al., 2009], West Nile virus [Krishnan et al., 2008], DENV [Sessions et al., 2009], and drosophila C virus [Cherry et al., 2005].

miRNA Profiling

miRNAs [Lee et al., 1993] play important roles in the control of stress signaling [Mendell and Olson, 2012], metabolism [Rottiers and Naar, 2012], tumorigenesis [Chen, 2005], and viral–host interactions [Jopling et al., 2005; Lecellier et al., 2005]. High-throughput miRNA profiling technologies, including quantitative reverse transcription PCR-based methods, hybridization-based methods, RNA-seq, and pri- and pre-miRNA quantification [Pritchard et al., 2012] have greatly enhanced knowledge regarding the role of miRNA in viral–host interactions (Fig. 1F). miRNA profiling technologies combined with messenger RNA (mRNA) profiling have provided new insight into HCV–host interactions. Investigation of miRNAs and mRNAs involved in HCV infection identified 43 differentially expressed miRNAs and 6,850 differentially expressed mRNAs expression levels of which were changed during HCV infection [Liu et al., 2010; Steuerwald et al., 2010]. These altered expression levels of miRNAs and mRNAs were involved in metabolism, cell growth, apoptosis, and cytokine/chemokine pathways, and in the progression of HCV-induced chronic hepatitis. Another study revealed 10 miRNAs that were downregulated in Hep-394 cells with 23 miRNAs upregulated [Braconi et al., 2010]. The identified miRNAs and their putative targets may be used as the basis for anti-HCV therapies, suggesting that combined miRNA and mRNA profiling may represent a novel approach to understand HCV infection and design new anti-HCV strategies.

Antisense inhibitors of miRNA function, that are biovailable in vivo, e.g., antagomirs, represent a good starting point for the development of miRNA inhibitory drugs [Krutzfeldt et al., 2005; Gottwein et al., 2007]. However, antisense inhibitors targeting cellular miRNAs have been predicted to have side effects, as these inhibitors could also disrupt cellular functions of these miRNAs. Moreover, like treatment with other antiviral drugs, the virus may become resistant, e.g., through mutation of the viral miRNAs or viral binding sites for cellular miRNAs [Gottwein and Cullen, 2008]. In this case, miRNA-based therapeutics would be needed to be used in combination with other antiviral drugs.

NEW INSIGHTS OF INFECTOMICS IN ANTIVIRAL DRUG DISCOVERY

Because of their relatively small genome, viruses must interact with host-encoding proteins to "hijack" host cellular signaling pathways and cellular processes, e.g., apoptosis, autophagy, and metabolism, to evade the host defense system and create a suitable microenvironment for their rapid replication [Bowie et al., 2004; Iannello et al., 2006; Galluzzi et al., 2010; Kaminskyy and Zhivotovsky, 2010]. Many groups have thus focused on host perspective to identify host factors involved in viral infection using system or network biology approaches have been used in studying viral infection and many cellular signaling pathways [de Chassey et al., 2008; Pauli et al., 2008; Shapira et al., 2009; Jia et al., 2010].

Signaling Pathways Involved in Viral–Host Interactions

Using high-throughput technologies (Table 1), new insights into virus–host interactions have occurred that suggest that host cellular signaling pathways may be hijacked or promoted by virus infection, making viral–host interactions more complex than previously thought. Receptors on the cell membrane, adaptor molecules in the cytoplasm, and nuclear transcription factors [Dai et al., 2011] represent three categories of host signaling molecules that play important roles in virus–host interactions [DeLarco and Todaro, 1976; Albritton et al., 1989; Doria et al., 1995; Choe et al., 1996; Yoneyama et al., 1998; Waris et al., 2001; Hemmi et al., 2004; Perry et al., 2004] (Fig 2). The phosphoi-

TABLE 1. A summary of new approaches used in infectomics

Approaches	Characteristics	Viruses been studied	References
MS-based proteomics	1. Investigate high degree complexity of protein 2. Research low abundance of some proteins 3. Identify the observed proteins	HCV	[Choi et al., 2004; Kou et al., 2006]
		HIV-1	[Jayasuriya et al., 2002]
		Dengue virus	[Pattanakitsakul et al., 2007; Higa et al., 2008]
		Influenza virus	[Williams et al., 2008; Schwahn et al., 2010]
		SARS-CoV	[Zeng et al., 2004; Jiang et al., 2005]
		RSV	[Brasier et al., 2004; Munday et al., 2010]
Activity-based protein profiling	1. Determine the changes in the catalytic state of enzymes in complex proteomes 2. Ascribe previously unknown enzymatic functions to proteins 3. Effective in targeting enzyme families with known covalent inhibitors	HCV	[Singaravelu et al., 2010; Blais et al., 2010a,b]
		HPV-1	[Rolen et al., 2006]
		HHV	[Lu et al., 2005]
		SARS-CoV	[Shah et al., 2010]
		HSV-1	[Kattenhorn et al., 2005]
		H1N1 virus	[Marnett et al., 2004]
Microarray analysis	1. Multiplex lab-on-a-chip 2. 2D array on a solid substrate 3. Genetic analysis, mRNA level analysis, and protein level analysis 4. A large number of targets can be analyzed in massive parallel measurements 5. Low sample consumption	HBV and HCV	[Iizuka et al., 2002]
		H1N1 virus	[Childs et al., 2009]
		HIV	[Geiss et al., 2000; Izmailova et al., 2003; Khodakov et al., 2008]
		ASFV	[Zhang et al., 2006]
		Parvovirus B19	[Kerr, 2005]
		Human influenza A and B viruses	[Li et al., 2001]
Yeast two-hybrid assays	1. Low-tech, without sophisticated equipment 2. Widely used in study protein–protein interaction 3. Scalable and time saving	HCMV	[To et al., 2011]
		HCV	[Kittlesen et al., 2000]
		Dengue virus	[Khadka et al., 2011]
		Stomatitis virus	[Moerdyk-Schauwecker et al., 2011]
		Influenza virus	[Sharma et al., 2011; Tafforeau et al., 2011]
		HTLV	[Simonis et al., 2012]
RNAi screening	1. Rapid, unbiased, and large scale 2. Independent of any preconceived models 3. Independent assumptions about gene functions	Influenza virus	[Karlas et al., 2010]
		HIV	[Brass et al., 2008; König et al., 2008]
		West Nile virus	[Krishnan et al., 2008]
		Dengue virus	[Sessions et al., 2009]
		DCV	[Cherry et al., 2005]
miRNA profiling	1. High-throughput technology 2. Can be combined with mRNA profiling 3. Expressive	HCV	[Braconi et al., 2010; Steuerwald et al., 2010]
		MHV-68	[Zhu et al., 2010]
		Adenovirus	[Qi et al., 2010]
		Enterovirus	[Cui et al., 2010]

HHV, human herpes virus.

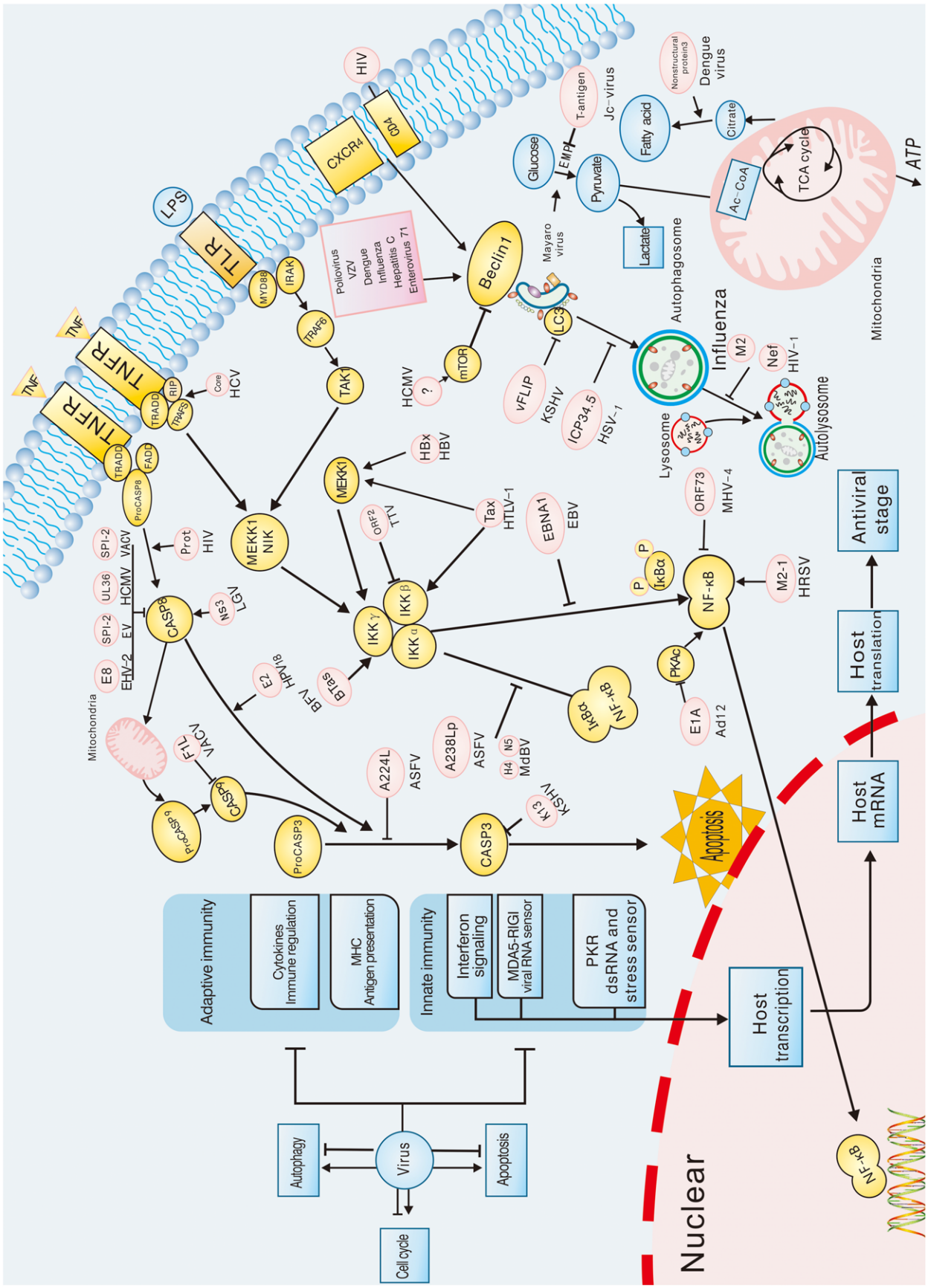
TNF
TNFR
TRADD
FADD
ProCASP8
RIP
TRAF2
Core
HCV
LPS
TLR
MYD88
IRAK
TRAF6
TAK1
MEKK1
NIK
Prot
HIV
SPI-2
UL36
HCMV
VACV
EV
EHV-2
E8
CASP8
NS3
LGV
Mitochondria
F1L
VACV
CASP9
ProCASP9
ProCASP3
CASP3
A224L
ASFV
A238Lp
ASFV
N5
H4
MdBV
K7.3
KSHV
E2
BFV
HPV8
BTas
IKK γ
IKK β
IKK α
MEKK1
HBx
HBV
ORF2
TTV
Tax
HTLV-1
EBNA1
EBV
IκBα
P
NF-κB
ORF73
MHV-4
M2-1
HRSV
PKAc
E1A
Ad12
Akt
NF-κB
HIV
gp41
CXCR4
Poliovirus
VZV
Dengue
Influenza
Hepatitis C
Enterovirus 71
Beclin1
mTOR
HCMV
?
vFLIP
KSHV
ICP34.5
HSV-1
LC3
Mayaro virus
Glucose
EMP
Pyruvate
Lactate
T-antigen
JC virus
Fatty acid
Nonstructural protein3
Dengue virus
Citrate
Ac-CoA
TCA cycle
ATP
Mitochondria
Autophagosome
Lysosome
Autolysosome
Influenza
M2
Nef
HIV-1
Apoptosis
Host transcription
Host mRNA
Host translation
Antiviral stage
Nuclear
NF-κB
Adaptive immunity
Cytokines
Immune regulation
MHC
Antigen presentation
Innate immunity
Interferon signaling
MDA5-RIGI viral RNA sensor
PKR dsRNA and stress sensor
Virus
Autophagy
Apoptosis
Cell cycle

Fig. 2. Overview of host signaling and cellular processes involved in viral infection. Integrated signaling networks functionally regulate the cellular processes of viral infection; implicated signaling pathways include NF-κB pathway, apoptotic pathway, autophagic pathway, and glucose metabolic pathway. Lines with an arrowhead indicate functional activation. Lines with a blunt end indicate functional inhibition. Ad12, human adenovirus 12; ATP, adenosine-5′-triphosphate; BFV, bovine foamy virus; CASP, capsase; CD4, cluster of differentiation 4; CXCR4, chemokine (C-X-C motif) receptor 4; EHV, equine herpes virus; EMP, Embden–Meyerhof–Pamas pathway; EV, ectromelia virus; FADD, Fas-associated protein with death domain; HCMV, human cytomegalovirus; HPV, human papillomavirus; HRSV, human respiratory syncytial virus; ICP34.5, neurovirulence factor infected cell protein 34.5; IκBα: nuclear factor kappa B inhibitor alpha; IKK, IκB kinase; IRAK, interleukin-1 receptor-associated kinase; LC3, microtubule-associated protein 1 light chain 3; LGV, Langat virus; LPS, lipopolysaccharide; MDA5, melanoma differentiation-associated gene 5; MdBV, microplitis demolitor bracovirus; MEKK1, mitogen-activated protein kinase/ERK kinase kinase-1; MHC, major histocompatibility complex; mTOR, mammalian target of rapamycin; MYD88, myeloid differentiation primary response gene 88; NIK, NF-κB inducing kinase; NS3, nonstructural protein 3; PKAc, protein kinase A catalytic subunit; PKR, double-stranded RNA-activated protein kinase; ProCASP, procaspase; Prot, gag-pol polyprotein; RIGI, cytoplasmic retinoic acid-inducible gene I; RIP, receptor-interacting protein; SPI-2, serine proteinase inhibitor 2; TAK, transforming growth factor β–activated kinase; TLR, toll-like receptor 1; TNF, tumor necrosis factor; TNFR, TNF receptor; TRADD, TNF receptor type 1-associated death domain protein; TRAF, TNF receptor-associated factor; UL36, uncharacterized protein 36; VACV, vaccinia virus; vFLIP, viral Fas-associated death domain-like IL-1β-converting enzyme inhibitory protein; VZV, varicella-zoster virus. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

nositide 3-kinases, a family of lipid kinases, are involved in intracellular signaling cascades that play essential roles in apoptosis, autophagy, and tumorigenesis [Wong et al., 2010; Bartholomeusz and Gonzalez-Angulo, 2012]. Influenza virus can interrupt this pathway to create a suitable environment for replication. Over the past few decades, nearly all classical signaling pathways were reported to participate in viral infections. Transforming growth factor-β (TGF-β) signaling initiates its cascades by binding to specific cell surface receptors that have intrinsic serine/threonine kinase activity [ten Dijke and Hill, 2004] and can modulate HCV infection [Lee et al., 2002; Rowan et al., 2008]. For virus-induced tumorigenesis, hepatitis B virus X protein and liver cancer-derived hepatitis C virus core proteins play essential roles in shifting TGF-β signaling from tumor suppression to tumorigenesis and epithelial-mesenchymal transition [Battaglia et al., 2009; Murata et al., 2009]. The nuclear factor kappa B (NF-κB), a family of transcription factors, plays a key role orchestrating innate and acquired host immune responses to pathogen infection [Rahman and McFadden, 2011]. Bovine foamy virus can activate the NF-κB pathway via action of its transactivator and enhanced viral transcription [Wang et al., 2010]. Other viral proteins involved in NF-κB activation include Tax of HTLV-1. Viral proteins can inhibit the NF-κB pathway and include Epstein-Barr nuclear antigen 1 and open reading frame 2 of Torque teno virus. Kaposi's sarcoma-associated herpes virus (KSHV) encodes a miRNA that controls viral replication by activating the NF-κB pathway demonstrating an important role of KSHV miRNAs in regulating viral latency and lytic replication via manipulation of the host survival pathway [Lei et al., 2010]. This suggests that not only the viral proteins but also miRNAs could participate in the regulation of host signaling pathways.

Cellular Processes Participated in Viral–Host Interactions

Virus–host interactions have indicated that cellular processes play important roles in viral infection. Autophagy, which can be divided into three categories: microautophagy, chaperone-mediated autophagy, and macroautophagy [Cecconi and Levine, 2008], have been linked to both innate and adaptive immunity versus viral infection [Lee and Iwasaki, 2008; Orvedahl and Levine, 2009]. Autophagy can be both activated [Ait-Goughoulte et al., 2008; Alavian et al., 2011] or inhibited [Zhou and Spector, 2008; Gannagé et al., 2009] by viral infection. In turn, autophagy can also promote [Lee et al., 2008] or inhibit [Orvedahl et al., 2010] viral infection. Therefore, unraveling the role of autophagy during viral infection is important for targeting autophagy and may provide new strategies for antiviral therapies. Apoptosis and glucose metabolism are also involved in viral infection. Noch et al., 2012] demonstrate the role of JC virus (John Cunningham virus) T-antigen in regulating glucose metabolism in brain tumor cells. T-antigen was downregulated by 2-deoxy-D-glucose, 6-aminonicotinamide, and oxythiamine. T-antigen can also modulate expression of the glycolytic enzyme, hexokinase 2, and the pentose phosphate enzyme, transaldolase 1 Epstein-Barr virus can inhibit induction of a pro-apoptotic B-cell lymphoma 2 homology 3-only containing protein (NOXA) by ionomycin inhibiting apoptosis in B cells [Yee et al., 2011]. In addition, HCV NS4B protein, a component of a membrane-associated cytoplasmic HCV replication complex [Hugle et al., 2001], has been reported as an inducer of apoptosis via a mitochondrila pathway [Zhao et al., 2012]. These findings suggested that cellular processes of the host cells could be altered during viral infection, which could contribute to the development of new

strategies to target these processes for combating viral infections (Fig. 2).

CONCLUSION

With the many novel technology platforms becoming available, omics approaches have been widely used in drug discovery. Studies based on genomics and proteomics approaches have established the foundation of network-based investigations for viral–host interactions, creating opportunities for the development of novel antimicrobial agents. Six infectomics technologies commonly used for drug discovery have been described in this review: MS-based proteomics, ABPP, microarray analysis, YTH assays, siRNA screening, and miRNA profiling. Together, they have the potential to elucidate and integrate the dynamic interactions between microbial pathogens and their hosts during the development of infectious diseases. Chemical infectomics, which has the advantages of both high-throughput chemistry and infectomics, is an emerging method for validating drug targets and will revolutionize approaches to infectious diseases. The infectomics view provides: (i) a global detection and integrative dissection of microbial and host infectomes, that is critical to understanding the process of microbial pathogenesis and developing better diagnostic or therapeutic approaches for infectious diseases; (ii) a powerful tool to investigate microbial and human genomes to address the present crisis in antibiotic resistance; (iii) a new method of utilizing pharmacomes (lipid-based drug delivery systems) for optimal drug therapy; and (iv) the opportunity to exploit probiotics as ecological approaches to infectious diseases. With such tools, prevention and treatment of microbial infections will eventually enter an era when holistic solutions to health problems can be efficiently individualized.

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